

**TITLE**

CORAL TOXICITY RESEARCH FOR DETERMINING THRESHOLDS FOR DISPERSANT-  
USE NEBA CALCULATIONS

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**ABSTRACT (#2017-136)**

The use of dispersants in close proximity to coral communities is generally not recommended, although Net Environmental Benefit Analysis (NEBA) of various response methods and priorities may override this principle. The NEBA calculation for use of dispersants near corals is a function of the relative effects of dissolved components of crude oil (alone) and components of chemically enhanced (dispersed) oil in the water column on corals. This is best determined by examining the toxicity (concentration and duration of exposure) of oil and dispersed oil to corals at the individual/organism and cellular levels. Unfortunately, this is a little studied area and very little coral toxicity information exists.

This paper describes ongoing multi-year research being conducted at Nova Southeastern University Oceanographic Center to fill the coral toxicity information gap and determine toxicity thresholds for individual corals. The research program was designed with inputs from an oversight committee and a broad group of collaborators from the response industry and toxicology communities. The output of the of the study is intended to be compatible with current and emerging predictive models such as NOAA's Chemical Aquatic Fate and Effects (CAFÉ)

database, which is used to estimate the fate and effects of thousands of chemicals, oils, and dispersants. An overview of previous and current research on hydrocarbon toxicity to corals will be presented, along with proposed guidelines for effective toxicity testing which conforms to standardized protocols and aids in comparison of results between studies and extrapolation to actual spills.

## **INTRODUCTION**

Coral reefs are typically found in coastal waters and can be adjacent to urban centers and/or major shipping lanes, which predisposes the potential for exposure to petroleum. In order to understand the impacts of oil pollution on coral reefs, it is necessary to increase our knowledge of the ways in which petroleum exposure may affect the basic element of a coral reef: the coral animal (Shigenaka 2001). The use of dispersants in close proximity to coral communities is generally not recommended, although Net Environmental Benefit Analysis (NEBA) of various response methods and priorities may override this principle. The NEBA calculation for use of dispersants near corals is a function of the relative effects of dissolved components of crude oil (alone) and components of chemically enhanced (dispersed) oil in the water column on corals. This is best determined by examining the toxicity (concentration and duration of exposure) of oil and dispersed oil to corals at the individual/organismic and cellular levels. Unfortunately, this is a little studied area and very little coral toxicity information exists.

## **STATE OF KNOWLEDGE**

Corals may be exposed to petroleum hydrocarbons via several pathways, and exposures can be characterized as either acute or chronic. Anthropogenic input of petroleum hydrocarbons to the marine environment stems from three main sources: extraction (releases from platforms

and pipelines, other operational discharges), transportation (releases from tanker, marine terminal and refinery operations), and consumption (runoff, recreational vessel and aircraft inputs) (Dodge et al. 1984, Burns and Knap 1989, NRC 2003). Terrestrial runoff is a significant component of anthropogenic hydrocarbon input, and coastal urban expansion increases the consumption impact on shallow coastal ecosystems. Acute exposures typically result from large spills originating from extraction or transportation sources which have immediate short-term effects, and exposure levels are related to proximity and duration of the spill (Dodge et al. 1984, NRC 2003, Haapkylae et al. 2007, Al-Dahash and Mahmoud 2013). Chronic exposures result from continuous exposure to small amounts of oil over long periods of time, typically from natural seeps and other point or non-point sources such as leaking pipelines, production discharges, atmospheric fallout and terrestrial runoff. As chronic exposures can result in a cascade of biological consequences, this type of oil pollution is often considered a larger threat than many types of acute exposures (Loya and Rinkevich 1980, Capuzzo 1987, NRC 2003).

Oil spill incidents in the marine environment provide an opportunity to assess impacts of oil exposure on organisms, and analysis of acute and chronic exposure on coral reefs indicate a variety of observed effects on coral. While no detectable impacts on coral were found after the Gulf War oil spill (Vogt 1995), other spills have resulted in major impacts related to hydrocarbon exposure (Burns and Knap 1989). Decreases in abundance, diversity, and coral cover have been observed, and tissue loss and increased coral mortality have been found after multiple acute and chronic exposures (Fishelson 1973, Bak 1987, Cubit et al. 1987, Guzmán et al. 1991, Jackson et al. 1989, Guzmán et al. 1994). Sublethal effects on corals after hydrocarbon exposure include decreased growth rate, bleaching, tissue swelling, mucous production and bacterial infections (Jackson et al. 1989, Guzmán et al. 1991, Guzmán et al. 1994, Green et al. 1997, White et al.

2012). Reproductive impacts such as decreased fecundity, gonad size, ova per polyp, planula per coral head and reduced settlement are found after chronic exposure to hydrocarbons (Rinkevich and Loya 1977, Guzmán and Holst 1993). Encrusting or massive species are typically less sensitive than branching species (Fishelson 1973, Bak 1987). Cellular and metabolic alterations including increased protein/lipid ratios, altered protein metabolic condition and increased mitochondrial chaperoning, have also been found following both chronic and acute exposures (Downs et al. 2006, Downs et al. 2012). Hydrocarbon exposure also resulted in immediate polyp retraction and sclerite enlargement in deep sea corals (White et al. 2012).

Field experiments exposing coral to oil *in situ* provide a controlled situation to monitor the effects of oil on reef corals, while constraining additional variables that can limit comparability between studies. Such experiments, while limited, enable the collection of baseline data, and control of exposure parameters. The TROPICS experiment found that exposure to dispersed oil initially resulted in significant reductions in coral cover and growth, with full recovery of all corals with regard to growth, sclerochronology, and coral cover after 10 years (Ballou et al. 1987, Ballou et al. 1989, Dodge et al. 1995). Branching species have been found to demonstrate a greater affinity for oil compared to massive or encrusting forms, with tissue damage occurring if oil adheres to the coral's surface (Johannes et al. 1972).

Of the research conducted on the effects of hydrocarbons on corals, laboratory experiments are the most numerous, and permit examination of the effects of petroleum hydrocarbons and dispersants on corals and their life stages under controlled, precise conditions. Laboratory experiments also provide the opportunity to calculate specific endpoints, such as the concentration lethal to 50% of the population ( $LC_{50}$ ), which is often used as a measure of toxicity for chemical compounds. However, the use of a wide variety of bioassay conditions and

exposure durations can result in high variability in calculated effect between experiments. Relative mortality was found to be highly variable following exposure to oil or WAF, from observations of no significant mortality to tissue flaking or rupture (Reimer 1975, Elgershuizen and De Kruijf 1976, Cohen et al. 1977, Peters et al. 1981, Wyers et al. 1986, Shafir et al. 2007, DeLeo et al. 2016). Sublethal changes included breakdown of polyp pulsation synchrony, reduced polyp retraction or extreme elongation, abnormal feeding and stimulus reactions, and mouth opening with exposed actinopharynx and mesenterial filament extrusion (Reimer 1975, Cohen et al. 1977, Ducklow and Mitchell 1979, Neff and Anderson 1981). Changes in cellular architecture, shifts in metabolic homeostasis, decreased photosynthetic yield, increased symbiont extrusion and alterations to mucous bacteria were also observed (Reimer 1975, Ducklow and Mitchell 1979, Neff and Anderson 1981, Peters et al. 1981, Mercurio et al. 2004, Rougee et al. 2006). Most effects were temporary, with a return to normal after a recovery period.

Exposure to petroleum during any stage of reproduction has been found to substantially alter reproductive output. Hydrocarbon exposure significantly inhibited fertilization and produced unusual embryonic development and disruption of cell membranes (Mercurio et al. 2004). Exposure to oil or oil WAF resulted in premature larval release, reduced or delayed settlement, less gonads per polyp, metamorphic alterations, and increased mortality of larvae (Loya and Rinkevich 1979, Rinkevich and Loya 1979, Te 1991, Kushmaro et al. 1997, Epstein et al. 2000, Goodbody-Gringley et al. 2013, Hartmann et al. 2015, Negri et al. 2016).

Exposure to dispersants or dispersed oil produced greater effects when compared to oil alone, and in most cases resulted in enduring damage with poor recovery. Sub-lethal behavioral changes were observed, including tentacle retraction, nematocyst discharge, feeding inhibition and reduced tactile response (Lewis 1971, Elgershuizen and De Kruijf 1976). An initial

reduction in carbon fixation and photosynthetic product uptake was found after exposure to dispersed oil, which was not observed after exposure to oil alone (Cook and Knap 1983). Tissue rupture and increased mortality were common, with dispersed oil exposure resulting in higher mortality than oil alone (Eisler 1975, Shafir et al. 2007, DeLeo et al. 2016). Effects of dispersed oil on reproduction included inhibition of fertilization, metamorphosis and settlement, as well as structural and behavioral anomalies of planula larvae. (Epstein et al. 2000, Lane and Harrison 2000, Negri and Heyward 2000, Goodbody-Gringley et al. 2013). The effects of petroleum PAHs on corals have also been evaluated. Single hydrocarbons (phenanthrene and 1-methylnaphthalene) significantly reduce calcium deposition following rapid accumulation and slow depuration; physical condition and cellular architecture was significantly affected after 48 h (Neff and Anderson 1981). Benzo(a)pyrene and a 13 PAH mixture produced cellular changes consistent with xenobiotic detoxification (Ramos and Garcia 2007, Woo et al. 2014).

Overall, oil and dispersed oil have been found to have significant effects on coral health in the laboratory. Field experiments provide a unique opportunity to assess the effects of hydrocarbons on corals *in situ*, and permit assessment of baseline data which assists in separation of oil impacts from other stressors. Coral gametes and larvae have been found to be more sensitive to hydrocarbon exposure compared to their adult stage; this early life stage sensitivity coupled with the buoyant nature of gametes and larvae increases the potential for negative impacts of oil exposure if a spill occurs during spawning season.

## **HYDROCARBON TOXICITY TESTING CHALLENGES**

Aquatic toxicity refers to the effects of a compound to organisms living in the water and is usually assessed with organisms which represent the three trophic levels, i.e. vertebrates (fish), invertebrates (crustaceans), and plants (algae). Aquatic toxicity tests allow evaluation of hazards

to aquatic life under standardized, defined exposures and durations, providing qualitative and quantitative data on adverse effects from a toxicant. Tests are typically run using selected organisms with ecologically relevant sensitivity to toxicants and a well-established literature background. Data from toxicity tests provide a database that can be used to assess the risk associated with a specific toxicant, and are used to estimate relative net environmental impact through evaluation of the potential for damage to an aquatic environment.

Establishing dose response relationships is key in quantifying toxic effects. These endpoints include both acute and subacute effects that are measured at each concentration in a gradient. For petroleum and associated response chemicals, toxicity tests are used to assess relative sensitivity of multiple test species. Species sensitivity distributions are used to compare hazard of different dispersants/oils to a test species, compare hazard of oil with and without dispersant, support regulatory approval of response chemicals, screening of potential response agents, and establishment of preapproved areas for dispersant use. Most importantly, such data is employed to inform decisions regarding spill management. However, toxicity testing with complex hydrocarbon mixtures presents unique challenges. The tremendous variability in composition between oils translates to a lack of comparability in toxicity data from experiments conducted with different oils. Each oil is a unique mixture comprised of numerous hydrocarbons from different classes, with varying degrees of volatility and water solubility, resulting in compositional changes relative to oil loading (Redman and Parkerton 2015). This presents significant challenges in interpreting and quantifying exposure concentrations, as concentration and solubility influence toxicity.

Exposure media used in toxicity testing with oils generally falls into two categories, physically dispersed oils and chemically dispersed oils. Physically dispersed oil in the

environment means oils that naturally dispersed or entrained in the water column by wind, waves, and currents. In the laboratory, this means media that is prepared by physical processes, through controlled mixing energy. With physically dispersed oils, toxicity is related to solubility and considerations include the oil type (weathered vs fresh), dispersion type (no vortex or percent vortex), mixing duration and settling time. For chemically dispersed oils, toxicity generally depends on the composition and concentration of dissolved oil, and is related to increased rates at which soluble components and droplets enter the water column. Considerations include dispersant type, oil loading (dispersants enhance partitioning into water column, and the same exposure concentration is generated at lower oil loadings), dispersant to oil ratio, mixing duration and settling time. Exposure type is a key consideration as well, and there are two main types of exposure protocols, constant and declining exposures. Constant exposures can take the form of static non-renewal, static renewal, and continuous flow-through, and may occur at multiple durations (commonly 24, 48, and 96 h); shorter exposures may provide better estimates of effects in the field. Declining exposures are used to better reflect short exposures characteristic of spill events. They generally use a standard test apparatus and standard rates of dilution (Singer et al. 2000, Aurand and Coelho 2005).

Endpoint determination relates to experimental design, and can be generally classified as acute or subacute. For  $LC_{50}$  determination, the acute endpoint must be clearly defined and relevant to your test species. Subacute endpoints ( $EC_{50}$ , the median effect concentration) relate ecological effects to durations and concentrations that are more realistic to real-world scenarios but may still have significant impact. These span a wide range of resolution, from physical changes in behavior to cellular and subcellular changes, and must again be well defined and relevant to the species tested. Another key consideration is the appropriate analysis of results;



specific guidelines exist for determining how best to analyze specific types of data (Bejarano et al. 2014, Redman and Parkerton 2015).

Practical considerations in toxicity testing should also include the nature and requirements of the test organism, such as the size of organism and consequent needed tank size, lighting (key for photosynthetic organisms, but must consider phototoxicity and photodegradation), temperature and salinity (of seasonal importance), depth, pressure, and nutrition (Bejarano et al. 2014, Redman and Parkerton 2015). The source and pre-test handling (filtration) of the water or seawater may affect some organisms and can impact the stability of exposure concentrations (by removal of microorganisms). Different species differ in their susceptibility to chemicals, probably due to differences in many factors, such as metabolic rate, excretion rate, genetic factors, dietary factors, age, health and stress level of the organism. The life stage of the organism tested must also be considered, as early life stages may be more sensitive than adults. Specific water quality requirements (pH, DO, alkalinity, etc.) differ between organisms and between life stages; food inputs and excretion during the test may significantly alter these parameters and confound results. Droplets in the exposure media can be important, as aquatic organisms are exposed to not only dissolved oil fractions which are taken up across membranes, but are also exposed to oil droplets which can be ingested or coat body surfaces. Pre-test storage methods of test oils and dispersants can also impact hydrocarbon composition in the exposure media (Redman and Parkerton 2015).

## **LIMITATIONS OF PREVIOUS CORAL RESEARCH**

Variability in toxicant, bioassay conditions, species and other methodological disparities can prevent comprehensive conclusions regarding the toxicity of hydrocarbons to corals during oil spills. Spill studies often lack quantitative baseline coral data needed to assess the degree of

change, or have not quantified hydrocarbon concentrations during the spill. This prevents the comparison of results between spills, and partially explains the variation in observed effects.

Field and laboratory experimental exposures aimed at quantifying the effects of petroleum pollution on corals have been designed to allow researchers the ability to control exposures and compounding factors. Conflicting results of a limited number of field exposure experiments were likely related to differences in oil type, dispersant used, species tested, or the physical conditions during the spills. Laboratory experiments are designed to limit differences between exposure scenarios and provide a means of comparing toxicity between different corals and oils. Though lab studies may use environmentally unrealistic concentrations or exposure durations, they are needed to assess relative species sensitivity and to provide essential information for use in toxicological models. To date, previous laboratory research has utilized multiple species which is useful to interpreting species sensitivity differences, but adds complexity when comparing different toxicants.

A key issue in evaluating published toxicity data is the inclusion of measured concentrations in exposure media, as the application of toxicity tests is the comparison of threshold concentrations of oil with values measured in the field (Bejarano et al. 2014). There is often a lack of detailed exposure-response data in field, mesocosm and laboratory studies. Use of nominal concentrations can either over or underestimate the lethality of a toxicant, and large differences in toxicity estimates are reported between studies using measured vs nominal values. This is of particular importance when comparing the toxicity of chemically dispersed to physically dispersed oil. Nominal concentrations were used in 27 of the 46 laboratory experiments reviewed in Turner (2016). Concentrations were not specified for 4 studies; of the 15 experiments with measured concentrations, 6 used measured stock solutions then serially

diluted to treatment concentrations which were not measured. Serial dilutions are not recommended, as the heterogeneous nature of complex mixtures can result in variable exposures. The remaining 9 exposures utilizing measured concentrations were from 5 experiments (Peters et al. 1981, Cook and Knap 1983, Dodge et al. 1984, Knap 1987, Wyers et al. 1986, Goodbody-Gringley et al. 2013, Renegar et al. 2017). These studies provided measured concentrations for each treatment utilized, providing the most accurate estimate of toxic threshold concentrations, and the best opportunity to compare toxicity between studies.

The composition of exposure media is of similar importance to concentration. No two petroleum products are compositionally the same, and most studies lack quantitative chemical composition analyses, preventing extrapolation of results. Compositional differences are created by the variety of preparation techniques utilized (mixing energy, headspace volume, ratio of oil to dispersant), and the specific composition of the oil used (Singer et al. 2000, Aurand and Coelho 2005). Of the 49 laboratory experiments described in Turner (2016), 31 different toxicants were utilized, including 15 crude oils, 8 refined products, 7 PAHs or PAH mixtures, and an unspecified petroleum toxicant. Although the broad range of toxicants utilized provides a wide view of petroleum toxicity to corals, comparison of effects between studies are cautioned due to the compositional differences between toxicants.

The method of exposure and other bioassay conditions also influences the toxicity of petroleum products. Coral exposure in the studies to date have included floating oil, prepared WAF or WSF, and various methods of direct oil contact. Bioassay conditions vary from static to flow through exposures over various time periods. Increased toxicity is observed in static conditions compared to flow-through (Cohen et al. 1977), indicating that corals have increased sensitivity to static conditions probably related to compounding effects such as decreased oxygen

and increased waste products. Open exposure vessels have the potential to allow volatile loss, therefore closed vessels which prevent such loss are associated with higher mortality (Te 1991).

### **NSU CORAL HYDROCARBON TOXICITY STUDY**

The multi-year research program on hydrocarbon toxicity to shallow water corals at Nova Southeastern University Oceanographic Center is designed to fill the coral toxicity information gap and determine toxicity thresholds for individual corals. The research project was designed with inputs from an oversight committee and broad group of collaborators from the response industry and toxicology communities. This study seeks to provide new information on sensitivity of corals to oil and dispersed oil, determine where corals fit on the scale of sensitivity to hydrocarbons, link field studies with controlled laboratory experiments, and substantially add to the body of oil/dispersant knowledge and science. Initial focus was on development of novel toxicity testing protocols and metrics tailored to the unique nature of corals, which included higher resolution (cellular level) effects whilst generating data that was compatible with current and emerging models. The overarching goal is to build a foundation for effective decision-making should a spill potentially impact coral reefs.

To avoid many of the complications associated with using whole oils or dispersed oils, the toxicity protocol developed was based on modeling toxicity of multiple oils based on toxicity of individual hydrocarbons. As the additive toxicity of constituents is equivalent to the toxicity of whole oil, toxicity of individual hydrocarbons can be used to model effects of any oil. Experiments to date have included 1-methylnaphthalene and phenanthrene; planned work will include toluene as well as the water accommodated fraction (WAF) and chemically enhanced water accommodated fractions of (CEWAF) of oil/dispersed oil to refine the accuracy of the

model. Additional studies are planned to assess other species, life stages, and compounding variables such as season, light, and temperature.

The exposure protocols and coral evaluation metrics were first used in a range-finding study with 1-methylnaphthalene, and described in Renegar et al. (2017). Subsequent toxicity tests with 1-methylnaphthalene and phenanthrene used five hydrocarbon concentrations and a control, with four replicates per concentration and five corals per replicate. A continuous flow recirculating exposure system is used; each is fully sealed, with < 10% headspace when filled and running. The hydrocarbon is passively dosed via PDMS o-rings, which produce a consistent concentration over the exposure period regardless of loss processes. The exposure design includes a four week pre-exposure acclimation period for collection of baseline data, a 48 hour exposure period, and a four week post-exposure period to assess delayed effects and/or recovery. Hydrocarbon concentrations were tested fluorometrically at the beginning and end of the exposure period, and basic water quality parameters such as pH, DO, alkalinity, temperature, and nutrients are tested. To determine EC<sub>50</sub>, physical condition of the corals is evaluated throughout the pre exposure, exposure and post-exposure periods using a semi-quantitative four level scoring system with 0 being within normal limits and 3 being severely affected. Changes in coloration, polyp extension/retraction, tissue swelling, and mucus production in addition to mortality and partial mortality are considered. Growth rate and photosynthetic efficiency were also measured, and cellular changes are assessed histologically.

For 1-methylnaphthalene, the 48-hr EC<sub>50</sub> estimate generated from the color and condition data was 4543 µg L<sup>-1</sup> (95% C.L.s =3071–6547 µg L<sup>-1</sup> ), and the 48-hr LC<sub>50</sub> estimate was 6524 µg L<sup>-1</sup> (95% C.L.s =5659–7500 µg L<sup>-1</sup> ) (Turner 2016). The utility in determining toxic thresholds of single hydrocarbons is the ability to predict toxicity of other hydrocarbons and combinations

of hydrocarbons using models like the target lipid model (TLM). Specifically, the TLM is used to calculate a critical target lipid body burden, which indicates that any type 1 narcotic chemical reaching this concentration in tissue lipids will cause mortality in 50% of the population. The calculated body burden for *P. divaricata* based on 1-methylnaphthalene is 356  $\mu\text{mol}$  chemical/g lipid (Turner 2016). A subsequent experiment with phenanthrene generated a 48-hr  $\text{EC}_{50}$  estimate of 398.8  $\mu\text{g L}^{-1}$  (95% C.L.s = 374.7–424.4  $\mu\text{g L}^{-1}$ ) and a 48-hr  $\text{LC}_{50}$  estimate of > 637.8  $\mu\text{g L}^{-1}$  (or greater than the highest concentration tested, and near solubility limit in seawater). This supported the prediction of the TLM, which suggested that hydrocarbons with a higher molecular weight than 1-methylnaphthalene would not be lethal at or below their solubility.

The current research program on hydrocarbon toxicity to shallow water corals was very much designed with the end in mind; through collaboration between academia, government, industry and responders, this study seeks to bridge the gap between good science and relevance. The complex nature and solubility of oil and petroleum substances present challenges for toxicity testing, and working with corals in any capacity can be challenging. The exposure scenarios in this study were designed to consider the characteristics and requirements of corals, employing protocols and metrics specifically for corals which consider acute and sub-acute effects at different levels of resolution. The use of individual hydrocarbons circumvents some of the difficulties of working with whole oils and allows modeling of the effects of any oil.

A significant contribution of this study is development and application of a standardized toxicity testing protocol for adult scleractinian corals which considers coral response at multiple levels of resolution and is applicable to many coral species and test scenarios. This has generated new hydrocarbon toxicity data for shallow-water corals and demonstrated lethal and sub-lethal impacts of 1-methylnaphthalene and phenanthrene to a model coral species. Further

experimentation utilizing this testing protocol with other hydrocarbons in additional coral species will contribute to a more complete picture of hydrocarbon toxicity to scleractinian corals.

### **APPLICATION TO OIL SPILL PREPAREDNESS AND RESPONSE**

The sum of experimental results, when integrated into response support tools, will provide input to managers for the visualization, prediction, and understanding of oil impacts on the coral animal and related habitats at variable severity levels. This will allow determination of thresholds of acceptable/ unacceptable impact, and prediction of impact severity and choice of treatment based on expected impact. Different scenarios of coral impact can also be evaluated for various levels of acute, chronic and catastrophic exposures that can be used for making policy decisions. This research is designed with the end in mind, and represents a collaboration between this laboratory, government, industry and responders with the goal of bridging the gap between good science and relevance by providing data that fills existing knowledge gaps and is compatible with predictive models. The overarching goal of this research program is to build a foundation for effective decision-making should a spill potentially impact coral reefs. This applied science approach to a practical issue allows improvement in decision-frameworks for reaction, response and mitigation and provides much needed information to be used in Net Environmental Benefit Analysis (NEBA) or Spill Impact Mitigation Assessment (SIMA) of predicted impacts and response methods in coral reef environments following an oil spill.

### **RECOMMENDATIONS FOR FUTURE RESEARCH**

Although numerous studies have assessed petroleum toxicity to corals, methodological disparities have precluded comprehensive conclusions regarding the toxicity of hydrocarbons to corals. Overall, previous studies have found exposure effects ranging from no observed effect to

complete mortality; sub-lethal effects were prominent in some studies and absent in others, presumably due to differences in study design. Sufficient characterization of exposure media was lacking in most previous research, and interpretation of results often utilized nominal concentrations. These limitations prevent direct comparison of results and preclude practical application of most toxicological data to spill response and management.

Coral toxicological studies with hydrocarbons should limit the differences in exposure media preparation, exposure scenarios and bioassay conditions. Corals are benthic organisms as adults, and petroleum exposure scenarios should generally focus on the water soluble fraction that is bioavailable to the coral animal. The use of standardized media preparations and standardized bioassay protocols (tailored to the unique requirements of corals) greatly increases the potential for comparisons across studies (Aurand and Coelho 2005, Redman and Parkerton 2015). These practices, when coupled with descriptive compositional analyses and quantifiable chemistry, will facilitate extrapolation of results to real world situations and enhance decision-frameworks for response and mitigation should an oil spill occur near coral reefs.

## REFERENCES

- Al-Dahash LM, Mahmoud HM. 2013. Harboring oil-degrading bacteria: a potential mechanism of adaptation and survival in corals inhabiting oil-contaminated reefs. *Mar. Pollut. Bull.* 72, 364-374.
- Aurand D, Coelho G. 2005. Cooperative Aquatic Toxicity Testing of Dispersed Oil and the Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF). Ecosystem Management and Associates, Inc. Technical Report 07-03, 105 + Appendices.
- Bak R. 1987. Effects of chronic oil pollution on a Caribbean coral reef. *Mar. Pollut. Bull.* 18, 534-539.
- Ballou TG, Dodge RE, Knap AH. 1989. Effects of untreated and chemically dispersed oil on tropical marine communities: a long-term field experiment. *International Oil Spill Conference Proceedings: February 1989, Vol. 1989, No. 1*, pp. 447-454



- Ballou TG, Hess SC, Getter CD, Knap AH, Dodge RE, Sleeter TD. 1987. Final results of the API tropics oil spill and dispersant use experiments in Panama. International Oil Spill Conference Proceedings: April 1987, Vol. 1987, No. 1, pp. 634B-634B
- Bejarano AC, Clark JR, Coelho GM. 2014. Issues and challenges with oil toxicity data and implications for their use in decision making: a quantitative review. Environ. Toxicol. Chem. 33, 732-742.
- Burns KA, Knap AH. 1989. The Bahia Las Minas oil spill hydrocarbon uptake by reef building corals. Mar. Pollut. Bull. 20, 391-398.
- Capuzzo JM. 1987. Chapter 8: biological effects of petroleum hydrocarbons: Assessments from experimental results., in: Rabalais, D.F.B.a.N.N. (Ed.), Long-term Environmental Effects of Offshore Oil & Gas Development. Elsevier, New York, pp. 343-410.
- Cohen Y, Nissenbaum A, Eisler R. 1977. Effects of Iranian crude oil on the red sea octocoral *Heteroxenia fuscescens*. Environ. Pollut. 12, 173-186.
- Cook CB, Knap AH. 1983. Effects of crude oil and chemical dispersant on photosynthesis in the brain coral *Diploria strigosa*. Mar. Biol. 78, 21-27.
- Cubit JD, Getter CD, Jackson JBC, Garrity SD, Caffey HM, Thompson RC, Weil E, Marshall MJ. 1987. An oil spill affecting coral reefs and mangroves on the Caribbean coast of Panama. International Oil Spill Conference Proceedings 1987, 401-406.
- DeLeo DM, Ruiz-Ramos DV, Baums IB, Cordes EE. 2016. Response of deep-water corals to oil and chemical dispersant exposure. Deep Sea Res. (II Top. Stud. Oceanogr.) 129, 137-147.
- Dodge RE, Baca BJ, Knap AH, Snedaker SC, Sleeter TD. 1995. The effects of oil and chemically dispersed oil in tropical ecosystems : 10 years of monitoring experimental sites. Marine Spill Response Corporation, Washington, D.C.
- Dodge RE, Wyers SC, Frith H, Knap AH, Smith S, Sleeter T. 1984. The effects of oil and oil dispersants on the skeletal growth of the hermatypic coral *Diploria strigosa*. Coral Reefs 3, 191-198.
- Downs CA, Ostrander GK, Rougee L, Rongo T, Knutson S, Williams DE, Mendiola W, Holbrook J, Richmond RH. 2012. The use of cellular diagnostics for identifying sub-lethal stress in reef corals. Ecotoxicology 21, 768-782.
- Downs CA, Richmond RH, Mendiola WJ, Rougee L, Ostrander GK. 2006. Cellular physiological effects of the MV Kyowa Violet fuel-oil spill on the hard coral, *Porites lobata*. Environ. Toxicol. Chem. 25, 3171-3180.
- Ducklow HW, Mitchell R. 1979. Bacterial populations and adaptations in the mucus layers on living corals. Limnol. Oceanogr. 24, 715-725.
- Eisler R. 1975. Toxic, sublethal, and latent effects of petroleum on Red Sea macrofauna, International Oil Spill Conference. American Petroleum Institute, pp. 535-540.

- Elgershuizen J, De Kruijf H. 1976. Toxicity of crude oils and a dispersant to the stony coral *Madracis mirabilis*. Mar. Pollut. Bull. 7, 22-25.
- Epstein N, Bak RPM, Rinkevich B. 2000. Toxicity of third generation dispersants and dispersed Egyptian crude oil on Red Sea coral larvae. Mar. Pollut. Bull. 40, 497-503.
- Fishelson L. 1973. Ecology of coral reefs in the Gulf of Aqaba (Red Sea) influenced by pollution. Oecologia 12, 55-67.
- Goodbody-Gringley G, Wetzel DL, Gillon D, Pulster E, Miller A, Ritchie KB. 2013. Toxicity of Deepwater Horizon source oil and the chemical dispersant, Corexit(R) 9500, to coral larvae. PLoS One 8, e45574.
- Green A, Burgett J, Molina M, Palawski D, Gabrielson P. 1997. The impact of a ship grounding and associated fuel spill at Rose Atoll National Wildlife Refuge, America Samoa. US Fish and Wildlife Service, Pacific Islands Ecoregion.
- Guzmán HM, Burns K, Jackson JB. 1994. Injury, regeneration and growth of Caribbean reef corals after a major oil spill in Panama. Mar. Ecol. Prog. Ser. 105, 231-241.
- Guzmán HM, Holst I. 1993. Effects of chronic oil-sediment pollution on the reproduction of the Caribbean reef coral *Siderastrea siderea*. Mar. Pollut. Bull. 26, 276-282.
- Guzmán HM, Jackson JB, Weil E. 1991. Short-term ecological consequences of a major oil-spill on Panamanian subtidal reef corals. Coral Reefs 10, 1-12.
- Haapkylae J, Ramade F, Salvat B. 2007. Oil pollution on coral reefs: A review of the state of knowledge and management needs. Vie et Milieu 57, 95-111.
- Hartmann AC, Sandin SA, Chamberland VF, Marhaver KL, de Goeij JM, Vermeij MJ. 2015. Crude oil contamination interrupts settlement of coral larvae after direct exposure ends. Mar. Ecol. Prog. Ser. 536, 163.
- Jackson JB, Cubitt JD, Batista V, Burns K, Caffey HM, Caldwell RL, Garrity SD, Getter CD, Gonzalez C, Guzmán HM, Kaufmann KW, Keller BD, Knap AH, Levings SC, Marshall MJ, Steger R, Thompson RC, Weil E. 1989. Ecological effects of a major oil spill on Panamanian coastal marine communities. Science 243, 37-44.
- Johannes R, Maragos J, Coles SL. 1972. Oil damages corals exposed to air. Mar. Pollut. Bull. 3, 29-30.
- Knap AH. 1987. Effects of chemically dispersed oil on the brain coral, *Diploria strigosa*. Mar. Pollut. Bull. 18, 119-122.
- Kushmaro A, Henning G, Hofmann D, Benayahu Y. 1997. Metamorphosis of *Heteroxenia fuscescens* planulae (Cnidaria: octocorallia) is inhibited by crude oil: a novel short term toxicity bioassay. Mar. Environ. Res. 43, 295-302.
- Lane A, Harrison P. 2000. Effects of oil contaminants on survivorship of larvae of the scleractinian reef corals *Acropora tenuis*, *Goniastrea aspera* and *Platygyra sinensis* from the Great Barrier Reef, Proceedings of the Ninth International Coral Reef Symposium, Bali, 23-27 October 2000, pp. 403-408.

- Lewis JB. 1971. Effect of crude oil and an oil-spill dispersant on reef corals. *Mar. Pollut. Bull.* 2, 59-62.
- Loya Y, Rinkevich B. 1979. Abortion effect in corals induced by oil pollution. *Mar. Ecol. Prog. Ser.* 1, 77-80.
- Loya Y, Rinkevich B. 1980. Effects of oil pollution on coral reef communities. *Mar. Ecol. Prog. Ser.* 3, 180.
- Mercurio P, Negri AP, Burns KA, Heyward AJ. 2004. The ecotoxicology of vegetable versus mineral based lubricating oils: 3. coral fertilization and adult corals. *Environ. Pollut.* 129, 183-194.
- Neff JM, Anderson JW. 1981. Response of marine animals to petroleum and specific petroleum hydrocarbons. Applied Science Publishers, Ltd., London, UK.
- Negri AP, Brinkman DL, Flores F, Botté ES, Jones RJ, Webster NS. 2016. Acute ecotoxicology of natural oil and gas condensate to coral reef larvae. *Sci. Rep.* 6, 21153.
- Negri AP, Heyward AJ. 2000. Inhibition of fertilization and larval metamorphosis of the coral *Acropora millepora* (Ehrenberg, 1834) by petroleum products. *Mar. Pollut. Bull.* 41, 420-427.
- NRC. 2003. Oil in the Sea III: Inputs, Fates, and Effects. The National Academies Press, Washington, DC, USA.
- Peters EC, Meyers PA, Yevich PP, Blake NJ. 1981. Bioaccumulation and histopathological effects of oil on a stony coral. *Mar. Pollut. Bull.* 12, 333-339.
- Ramos R, Garcia E. 2007. Induction of mixed-function oxygenase system and antioxidant enzymes in the coral *Montastraea faveolata* on acute exposure to benzo(a)pyrene. *Comp. Biochem. Physiol., C: Toxicol. Pharmacol.* 144, 348-355.
- Redman AD, Parkerton TF. 2015. Guidance for improving comparability and relevance of oil toxicity tests. *Mar. Pollut. Bull.* 98, 156-170.
- Reimer AA. 1975. Effects of crude oil on corals. *Mar. Pollut. Bull.* 6, 39-43.
- Renegar DA, Turner NR, Riegl BM, Dodge RE, Knap AH, Schuler PA. 2017. Acute and subacute toxicity of the polycyclic aromatic hydrocarbon 1-methylnaphthalene to the shallow-water coral *Porites divaricata*: Application of a novel exposure protocol. *Environ. Toxicol. Chem.* 36, 212-219.
- Rinkevich B, Loya Y. 1977. Harmful effects of chronic oil pollution on a Red Sea scleractinian coral population, in: Taylor, D.L. (Ed.), *Proceedings*, 3rd International Coral Reef Symposium, Miami, FL, USA, pp. 585-591.
- Rinkevich B, Loya Y. 1979. Laboratory experiments on the effects of crude oil on the Red Sea coral *Stylophora pistillata*. *Mar. Pollut. Bull.* 10, 328-330.

- Rougee L, Downs CA, Richmond RH, Ostrander GK. 2006. Alteration of normal cellular profiles in the scleractinian coral (*Pocillopora damicornis*) following laboratory exposure to fuel oil. *Environ. Toxicol. Chem.* 25, 3181-3187.
- Shafir S, Van Rijn J, Rinkevich B. 2007. Short and long term toxicity of crude oil and oil dispersants to two representative coral species. *Environ. Sci. Technol.* 41, 5571-5574.
- Shigenaka G. 2001. Toxicity of oil to reef-building corals: A spill response perspective. U.S. Dept. of Commerce, National Oceanic and Atmospheric Administration, National Ocean Service, Office of Response and Restoration, Silver Spring, MD.
- Singer MM, Aurand D, Bragin GE, Clark JR, Coelho GM, Sowby ML, Tjeerdema RS. 2000. Standardization of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. *Mar. Pollut. Bull.* 40, 1007-1016.
- Te FT. 1991. Effects of two petroleum products on *Pocillopora damicornis* planulae. *Pac. Sci.* 45, 290-298.
- Turner NR. 2016. Quantifying the toxicity of 1-methylnaphthalene to the shallow-water coral, *Porites divaricata*, for use in the target lipid model. Nova Southeastern University, p. 119.
- Vogt I. 1995. Coral reefs in Saudi Arabia: 3.5 years after the Gulf War oil spill. *Coral Reefs* 14, 271-273.
- White HK, Hsing P-Y, Cho W, Shank TM, Cordes EE, Quattrini AM, Nelson RK, Camilli R, Demopoulos AWJ, German CR, Brooks JM, Roberts HH, Shedd W, Reddy CM, Fisher CR. 2012. Impact of the Deepwater Horizon oil spill on a deep-water coral community in the Gulf of Mexico. *Proc. Natl. Acad. Sci. USA* 109, 20303-20308.
- Woo S, Lee A, Denis V, Chen CA, Yum S. 2014. Transcript response of soft coral (*Scleronephthya gracillimum*) on exposure to polycyclic aromatic hydrocarbons. *Environ. Sci. Pollut. Res. Int.* 21, 901-910.
- Wyers SC, Frith HR, Dodge RE, Smith SR, Knap AH, Sleeter TD. 1986. Behavioural Effects of Chemically Dispersed Oil and Subsequent Recovery in *Diploria strigosa* (Dana). *Mar. Ecol.* 7, 23-42.