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The first decade of scientific insights from the Deepwater Horizon oil release

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Abstract

The 2010 Deepwater Horizon disaster remains the largest single accidental release of oil and gas into the ocean. During the 87-day release, scientists used oceanographic tools to collect wellhead oil and gas samples, interrogate microbial community shifts and activities, and track the chemical composition of dissolved oil in the ocean's interior. In the decade since the disaster, field and laboratory investigations studied the physics and chemistry of irrupted oil and gas at high pressure and low temperature, the role of chemical dispersants in oil composition and microbial hydrocarbon degradation, and the impact of combined oil, gas and dispersants on the flora and fauna of the deep-sea and the coastal environments. The multi-faceted, multi-disciplinary scientific response to the released oil, gas and dispersants culminated in better understanding of the environmental factors that influence the short- and long-term

29 fate and transport of oil in marine settings. In this Review, we summarize the unique aspects of the

30 Deepwater Horizon release and highlight the advances in oil chemistry and microbiology that resulted

31 from novel applications of emerging technologies. We end with an outlook on the applicability of these

32 findings to possible future oil releases in future deep-sea drilling locations and newly-opened high-

33 latitude shipping lanes.

34 **Key points**

35 • The Deepwater Horizon disaster was the largest single accidental release of oil and gas to the

36 ocean. Over 87 days, oil, gas and dispersants impacted 11,000 square kilometers of ocean surface

37 and 2000 km of coastline.

38 • Subsurface dispersant application was a unique characteristic of the DWH disaster. Empirical

39 observations, laboratory data and modeling efforts offer conflicting conclusions as to whether

40 dispersants reduced the sea surface expression of released oil.

41 • The DWH disaster was the first environmental application of emerging systems biology tools

42 based on microbial gene analysis. These tools included meta- and single-cell genomics,

43 metatranscriptomics, microarrays, and stable-isotope probing enrichments. They provided

44 unprecedented insights into the identity, structure, growth dynamics, succession, and overall

45 response of microbial communities to oil, gas and dispersant substrates released to marine

46 ecosystems.

47 • Advanced analytical chemistry technologies including ultra-high resolution Fourier-transform

48 ion cyclotron resonance mass spectrometry and comprehensive two-dimensional gas

49 chromatography provided novel information regarding source oil composition, biodegradation,

50 photochemical oxidation, water-column processes, accurate measurements of biomarkers, and

51 identification of oil weathering products irrespective of boiling point.

52 • The Gulf of Mexico coastline and deep ocean were contaminated with oil, gas and dispersants to

53 differing degrees. In many cases, coastal ecosystems recovered as predicted from previous oil

54 release studies, while in others, the disaster combined with other stressors to deleterious effect.

55 Examination of the disaster's impacts on the deep sea, and its ongoing recovery, continue.

56 • Insights from the first decade of DWH-related research underscore the need for integrated

57 analytical platforms and data synthesis to understand the complexities of the environmental

58 responses to oil, gas and dispersant release. Although conclusions from the DWH disaster may

59 not be fully applicable to the next spill, the spill science community should be ready to work

60   collaboratively across academia, industry and government during possible future oil releases in

61   the deep sea and high latitudes.

62   **Table of contents summary**

63   The Deepwater Horizon disaster was the largest accidental discharge of oil into the environment, and

64   was intensely studied during and after the event. In this Review, the subsequent advances made in oil

65   chemistry, dispersant application, and microbiology are discussed.

[H1] Introduction

The explosion of the Deepwater Horizon (DWH) drilling rig in the northern Gulf of Mexico on April 20, 2010 caused the deaths of 11 crew members and marked the onset of an uncontrolled oil and natural gas release from the damaged Macondo Well, located 80 km off the coast of Louisiana in the United States' (US) Bureau of Ocean Energy Management's Mississippi Canyon lease block 252 (MC-252) at a water depth of 1,500 meters (**Fig. 1**). The well was finally capped on July 15, 2010, by a team of academic and industrial engineers. Over the 87-day period, the uncapped well released 5.3×10^{11} g of oil (defined as hydrocarbons with $\geq$ 6 carbons that are liquid at 1 atm; REF[1]) and 1.7×10^{11} g of natural gas (hydrocarbons with $\leq$ 5 carbons that are gases at 1 atm; REF[1]) at high pressure (1.5×10^4 kPa) into the overlying water column, leading to extensive dissolution of hydrocarbons in the deep sea[2,3], referred to as the subsurface intrusion. The remainder of the hydrocarbons traveled to the sea surface, where 1.4-2.0×10^{11} g of the volatile compounds ($\leq$ n-C_{16} compounds) evaporated into the atmosphere within 3-10 h of surfacing[4-6] and 3.3-3.6×10^{11} g of the less-volatile compounds (> n-C_{16} compounds[4-6]) spread over $11 \pm 5 \times 10^3$ km^2 (REF[7]), reaching a total of 2×10^3 km of coastline along five Gulf of Mexico states[8] (**Fig. 1**). Approximately 2-20% of the total released hydrocarbons were deposited on the seafloor[9], primarily as oil residue. In response to the disaster, 2.9×10^6 and 4.1×10^6 L of chemical dispersants **[G]** were applied at the wellhead and to the sea surface, respectively[10], in efforts to reduce surface oil slicks and sequester oil in the deep sea, more than any other known application in history. In total, the DWH spill was unprecedented by almost any metric of marine oil discharge disasters, including volume and scale of hydrocarbon release, depth of the discharge, and application of surface and sub-surface dispersants.

The DWH disaster posed several scientific and logistical challenges, including where the oil and gas would travel after irruption from the wellhead, whether chemical dispersants could alter the oil and gas fate and transport without significant environmental impact and how affected ecosystems and their inhabitants—from the deep-sea to the coastal marshes— would respond to discharged oil and gas and the applied dispersants. The unique location (deep-sea) and oil-and-gas volume-spilled of this disaster rendered previous models and predictions of oil and gas fate poorly applicable, thus requiring a rapid shift in response planning and execution. Within weeks of the DWH explosion, researchers and responders realized that four zones would need to be examined for released oil, gas and dispersants: the subsurface where dissolved oxygen anomalies indicated a large intrusion of hydrocarbons was degraded by oxygen-consuming bacteria[3,11,12]; the surface where oil sheens and emulsions extended over 11,000 km^2

96 (REF[7]); the atmosphere where volatilized oil and gas were rapidly transported away from the crippled oil

97 rig[13], and the sea floor, where oil might sink due to weathering or ballasting[14,15].

98 These overarching challenges required observing and sampling the earliest stages of the oil discharge,

99 which is difficult as such disasters progress faster than most research can be mobilized[16]. Furthermore,

100 these challenges could not be addressed with the standard technologies at the time, which were mostly

101 prescribed by the Oil Pollution Act of 1990 (legislated partly in response to the 1989 *Exxon Valdez* spill)

102 and were obsolete because academic research on oil spills had stagnated. Nevertheless, the scientific

103 community responded quickly to the DWH disaster by altering field plans, and as a result, research

104 expeditions in the Gulf of Mexico were the first to detect[17,18] and map[12,19] the subsurface intrusions of oil

105 and gas travelling away from the wellhead. Academic and government scientists mobilized tools and

106 techniques repurposed from basic science uses, such as those used when studying natural oil and gas

107 seeps and the biogeochemical cycling of methane and other gases[20-23] (**Fig. 2**). Oceanographic

108 instrumentation was deployed to track and sample oil and gas flowing from the damaged well[1] and to

109 measure oil droplet sizes[24] and dissolved low-molecular weight components within the subsurface

110 plume[19,25]. During the DWH event, additional research expeditions collected surface and deep water to

111 assess microbial community dynamics[3,11], explore chemical transformations in oil and gas components[2]

112 and track the fate of chemical dispersants[26]. Government scientists at the US National Oceanic and

113 Atmospheric Administration (NOAA) mobilized overflights to assess air quality and hydrocarbons that

114 had volatilized to the atmosphere[13]. While early analyses of these samples and the follow-up field,

115 laboratory and modelling studies were funded initially by a combination of BP, the US National Science

116 Foundation (NSF; "RAPID" grants), the US Department of Energy (DOE) and the US National Oceanic

117 and Atmospheric Administration (NOAA), BP and other responsible parties paid $19 billion US towards

118 post-disaster research and recovery[27], including $500M for the Gulf of Mexico Research Initiative

119 (GoMRI). Scientific investigation following the DWH disaster has collectively culminated in over 5000

120 publications to date (January 2020, based on Google Scholar search with "Deepwater Horizon" and "oil

121 spill"), with research still ongoing. The breadth and diversity of scientific inquiry into the DWH disaster

122 enabled significant advances in oil chemistry and microbiology, but also shifted perceptions in these

123 fields about the knowledge gained from integration of complementary datasets to understand the

124 complexity of environmental perturbations.

125 In this Review, scientific questions raised by and results from the DWH disaster are discussed, with a

126 focus on the chemical and microbiological aspects of the disaster and ongoing recovery. The irruption,

127 dissolution, and microbial degradation of oil and gas in the deep-sea, which is inaccessible to typical oil

128 response procedures[3,5,12,19], are detailed. We then explore the impact of subsurface dispersant applications

129 on oil composition, the extent of hydrocarbon biodegradation and deep-water ecosystems, which were

130 previously unpredictable because prior usage was solely on the sea surface. Finally, we review

131 environmental factors that led to extensive weathering of the oil and gas over the 87-day duration of the

132 discharge, the broader environmental impacts of the spill, and future directions for oil disaster science.

133 **[H1] Subsurface oil, gas and dispersants**

134 During a deep-sea oil well blowout like the DWH disaster, 100°C hydrocarbon gas and oil irrupts at high

135 pressure into 4°C water[1]. Components of the released oil and gas follow different paths from the

136 wellhead, with some rising to the surface, and the remainder forming a deep-sea intrusion of oil and gas

137 at 1100 m water depth due to natural buoyancy[17,19]. Predicting the partitioning of irrupted oil and gas

138 between the subsurface intrusion layer and the sea surface is very difficult, as the distance and speed of

139 the transport depends on the physical properties of the surrounding seawater (which were known), the

140 oil-to-gas ratio and flow dynamics at the release point, and oil composition, droplet size, and buoyancy.

141 Existing models of oil droplet size distribution were based on surface water models and lab experiments,

142 and thus did not reflect deep-sea conditions; as a result, *in situ* observations were needed to constrain the

143 oil's fate. In one example, holographic imaging equipment developed for studying the morphologies and

144 distributions of marine micro- and mesozooplankton (the Video Plankton Recorder and the Holocam[28-30])

145 was adapted. Starting in early June (approximately six weeks after the DWH well blowout), oil droplets

146 and plankton at depth were successfully imaged with this equipment a few km away from the Macondo

147 well[24,30,31], providing oil droplet size distribution data that was previously unattainable and highlighting a

148 technology that can be used in future deep-sea spills[27]. Imaging data did not capture the oil droplet size

149 distribution at the wellhead, and thus subsequent modeling[32,33] and laboratory[34,35] efforts have focused on

150 understanding the role of oil-to-gas ratio, source geometry conditions and dispersant application on

151 release trajectories and droplet-size distributions.

152 Oil droplets can be categorized into three size categories, each with different buoyancies, but with

153 overlapping chemical compositions[5]. The largest oil droplets (greater than 0.3 mm diameter) exhibited

154 sufficient buoyancy to rise to the ocean surface, with rise times below four hours[5], transporting a mixture

155 of soluble and insoluble compounds to the sea surface where some volatilized into the atmosphere[13,36]

156 (**Fig. 3**). The medium-sized droplets (0.1 – 0.3 mm) ascended more slowly (rise times below 10 hrs), and

157 their composition was very sensitive to initial oil composition and release dynamics. For example,

158 moderately soluble hydrocarbons such as toluene, xylene, naphthalene and (methyl)cyclopentane

159 partitioned to the aqueous phase from all droplet sizes as a function of the droplet exposure time and

160 surface area-to-volume ratio, as well as their relative concentration in the source oil[5]. In contrast, the

161 smallest droplets (smaller than 0.1 mm) and soluble gases (methane, ethane, propane) lacked the

162 buoyancy to rise after being emplaced in deep-sea intrusions, although the degree of hydrocarbon

163 dissolution could not be measured due to challenges in separating oil droplets from the surrounding

164 waters[5]. The bulk of the released oil and gas (by mass) was retained in the deep-sea intrusions because of

165 the relatively high proportion of gases in the DHW irruption. However, reducing the sea surface

166 expression of released oil was a high priority during DWH mitigation efforts and thus responders were

167 motivated to disrupt the larger, faster-rising size classes of oil droplets into small droplets that would

168 remain in the deep-sea. At the same time, reducing droplet sizes and maximizing dissolution should

169 make oil and gas more accessible to microbial degradation[37].

170 Chemical dispersants were used by the DWH disaster responders to decrease the size of oil droplets[37].

171 Dispersants (such as Corexit 9500, the primary formulation used in the DWH disaster) are a mixture of

172 nonionic surfactants (~30% Tween and Span surfactants), an anionic surfactant (~20% dioctyl sodium

173 sulfosuccinate or DOSS) and solvent carriers (~50% petroleum distillates[38]). However, prior to the DWH

174 disaster, dispersants had been applied only to surface oil spills[37] with their use determined by wind

175 speed, wave height, and oil age and composition. Dispersants were used on the sea surface during the

176 DWH disaster, but they were also applied directly at the wellhead in the first deep-sea dispersant

177 application. The fate of these added dispersants was a public and scientific concern; after all, 7×10^6 L

178 dispersant were added to the deep-sea with unknown consequences. Because dispersants are a complex

179 mixture, specific components had to be analyzed separately to ascertain the lability and persistence of the

180 dispersants during and after the spill. After the disaster, DOSS was used to track the fate of the deep-sea

181 dispersants because it comprised a large and relatively constant fraction of Corexit and other dispersant

182 formulations and it was amenable to existing analytical protocols. Samples taken during and a few

183 months after the spill indicated that DOSS persisted in the subsurface intrusion and was not degraded[26],

184 contrary to expectations based on laboratory experiments performed at surface conditions[39]. These data

185 suggest that the chemical dispersants were not degraded appreciably in the deep sea in the aftermath of

186 the DWH disaster. Subsequent laboratory work showed that DOSS was less labile than the solvent

187 carriers in the dispersants under deep-sea conditions[39], and others have found minimal degradation of

188 DOSS at low temperatures[40]. Other surfactant components have been more challenging to detect and

189 quantify due to their isomeric diversity and amphiphilic [G] properties[41], but recent studies have shown

190 that these components were present in the subsurface intrusion layers (typically below 15 μg/L[42]) and are

191 degraded at deep-sea (4°C) temperatures[43].

192 Once it appeared that deep-sea dispersants were retained in the subsurface intrusion, the primary

193 question was whether this intervention had indeed shifted the oil-droplet size distributions as intended.

194 Unfortunately, empirical observations, laboratory data and modeling efforts offer conflicting conclusions

195 as to the efficacy of the dispersants in this regard. Operational tests during the DWH disaster noted oil

196 reduction at the sea surface during "on/off" experiments[44]. Modeling results are equivocal on whether

197 dispersants entrained additional oil and gas into the subsurface intrusion layers, with one group arguing

198 that they did[4] and another arguing that they did not[32,33]. The inconsistency of the modeling results may be

199 due to the challenges of accurately parameterizing the complex physics of the outflow at the wellhead,

200 including the impacts of initial droplet size distribution, churn flow[45], source pressure, orifice geometry,

201 and dispersant applicator type. Nevertheless, dispersant addition in the subsea appeared to impact sea

202 surface expression, fulfilling the first motivation of the response teams. Whether dispersants shifted the

203 droplet size distribution or enhanced oil degradation in the subsurface intrusion layer remains a matter of

204 debate.

205 **[H1] Microbial degradation of oil, gas and dispersants**

206 During and after the DWH disaster, most microbiology research focused on the subsurface intrusion

207 layer because of its novelty. Comparatively little data was collected on the microbes responding to oil in

208 surface waters in part because microbial degradation was a minor loss term, relative to the natural

209 processes of evaporation and photochemical weathering[46] and the responder oil mitigation efforts of

210 skimming and burning. The surface Gulf of Mexico has low concentrations of nutrients which prevented

211 bloom formation, although some biodegradation was observed during the spill[47]. Limited data indicated

212 an enrichment of *Cycloclasticus*, with minor contributions from organisms including *Alteromonas*,

213 *Colwellia*, and *Pseudoalteromonas* in the surface slick[48]. Enrichments showed that *Cycloclasticus* and

214 *Colwellia* were dominant phenanthrene-degraders, whereas *Alteromonas* dominated the naphthalene-

215 degrading community[49,50]. Some of these organisms were observed in pre-DWH studies in surface Gulf

216 waters[51,52].

217 Unlike surface waters, nutrients are abundant at depth in the Gulf of Mexico, setting the stage for

218 microbial blooms driven by the entrainment of hydrocarbon substrates defining the subsurface intrusion

219 layers. Microbial degradation was the only option available for oil mitigation in this environment because

220 perpetual darkness prevents photo-oxidation [G], and meaningful recovery of dilute oil from depth is

221 technically infeasible. The sudden influx of oil, gas, and dispersants enriched a community of

222 hydrocarbon-degrading organisms[11], the composition of which was influenced by the physical forms and

223 chemical composition of the oil-gas-dispersant mixtures. As the hydrocarbon composition in droplets and

224 dissolved phases changed[53], different microbes bloomed[54,55] following the succession pattern of

225 Oceanspirillales - *Colwellia* - *Cycloclasticus* - Methylophaga and Methylococcaceae - Flavobacteriaceae and

226 Rhodobacteriaceae[11,54,55] (**Fig. 4**). This succession was captured in groundbreaking detail through the first

227 effective application of combined systems biology tools to an environmental event, something was

228 previously prohibitively expensive and computationally unfeasible[56]. The use of metagenomics [G],

229 metatranscriptomics, [G] and other molecular biology tools on hundreds of samples offered a view into

230 the response of the hydrocarbon-degrading microbial community to released oil and gas over

231 environmentally relevant spatial (meters to kilometers) and temporal (days to weeks) scales[56].

232 In addition to enabling the rapid assessment of the identities of the responding microorganisms, the

233 biological data (including stable isotope probing [G], or SIP) provided insight into the microbial potential

234 for degradation of hydrocarbons in the hydrocarbon-contaminated deep sea (reviewed in REF[52]). Field-

235 based microbial community composition assessments[11] combined with metagenomics[57] and

236 transcriptome microarray data[11] noted a rapid enrichment of Oceanospirillales spp. (Gamma-

237 proteobacteria) within the subsurface intrusion. These organisms were the first to bloom and likely

238 degraded highly labile *n*-alkanes and cycloalkanes[57], based on metabolic potential reconstructed from

239 DNA sequences within field populations (metagenomes and single-cell amplified genomes)[57] and

240 incubations with ^{13}C-naphthalene[49]. Similarly, SIP incubations and single cell-amplified genome data

241 suggested that *Cycloclasticus* and *Colwellia* degraded the dissolved gases, propane and ethane[58,59], and

242 *Marinobacter* and *Alcanivorax* degraded *n*-hexadecane[49,50]. *Cycloclasticus* populations likely also

243 mineralized less labile hydrocarbons, such as the aromatic compounds of benzene, toluene, ethylbenzene,

244 and xylenes (BTEX)[58], based on the presence of genes encoding aromatic hydrocarbon degradation in SIP

245 incubations with ^{13}C-benzene. Lastly, *Cycloclasticus* collected from the deep sea contained the genetic

246 potential for various divergent monooxygenases, which are enzymes that oxidize alkyl functional groups

247 on hydrocarbons[59], and expressed those genes at high abundance in DWH-contaminated waters[60].

248   By late May and early June 2010, genes for methane oxidation were enriched in the subsurface intrusion,

249   suggesting an increase in methane- and methyl-degrading bacteria, such as *Methylomonas*, *Methylococcus*,

250   and *Methylophaga*, in the subsurface intrusions[2,55,60,61]. Finally, in late August and September 2010, bacterial

251   clades with scavenging members such as Flavobacteriaceae and Rhodobacteriaceae bloomed in the

252   subsurface intrusion, with likely roles in degrading residual hydrocarbons and complex organic matter

253   released by earlier microbial blooms[55]. The increase in transcript abundance was also higher than the

254   relative increase in bacterial cell numbers (factor of 2-3 cell number increase in subsurface intrusion

255   samples versus unaffected samples[11]), suggesting a broad ability of indigenous deep-sea Gulf of Mexico

256   bacteria to degrade hydrocarbons. In total, few bacteria were inhibited by the presence of oil and gas

257   (~5% of taxa showed decreased transcript expression in the intrusion layer relative to uncontaminated

258   samples[60]) and many common non-hydrocarbon degrading deep-sea bacteria, such as SAR11, did not

259   respond to the oil, gas, or dispersants[62].

260   The metabolic map and succession of hydrocarbon-degrading communities described above was

261   developed with gene-based analyses, including meta- and single-cell genomics, metatranscriptomics, and

262   microarrays. These tools were applied to field samples, stable-isotope probing enrichments, and cultured

263   isolates. Converting these data to rates of hydrocarbon degradation, however, is not straightforward.

264   Instantaneous rate measurements were possible for selected DWH components, most notably

265   methane[3,60,63], the largest component by mass[1], but most substrate degradation rates remain unknown[64].

266   Rate assays with chemical monitoring of substrates are challenging experiments, particularly when

267   microbial consortia (rather than monocultures) work synergistically to degrade released oil and gas, and

268   when the hydrocarbon substrates are present in complex mixtures, rather than in single additions.

269   Selecting microbes[65] (or groups of microbes) and their substrates[66] (or groups of substrates) is fraught

270   with potential artifacts; yet these experiments are critically needed to support predictive modeling of oil

271   degradation under different environmental scenarios. While indirect geochemical referencing provided

272   long-term degradation rates for some compounds[67], a fundamental challenge for future oil spills will be

273   to connect the intricacy of hydrocarbon-degrading metabolism, inferred from gene-based data, with the

274   complexity of the petroleum substrate.

275   The addition of chemical dispersants like Corexit 9500 impacted the response of the microbial community

276   to the DWH disaster, though the type and magnitude of the impact is unresolved[27], and the effects of the

277   dispersants depend on the specific oil compounds and on the bacterial species present[68-71]. Current

278   evidence indicates that the dispersant Corexit 9500 is effective at decreasing oil droplet size, leading to

279 increased microbial degradation of hydrocarbons relative to untreated oil, especially at higher

280 temperatures (such as 25°C) and/or when oil concentrations are in the low to sub-ppm range[40,72-76].

281 Investigators established mesocosms with uncontaminated deep Gulf seawater to explore the microbial

282 responses to oil, Corexit or both additions. *Colwellia* spp. were likely consumers of ethane and propane[58],

283 but were also enriched with oil and dispersed oil treatments, and dominated flocs, in mesocosms[39]. Other

284 mesocosms reported enrichment of *Colwellia* spp. within Corexit 9500 and dispersed oil treatments, but

285 not in oil-only treatments, where *Marinobacter* spp. were enriched instead[66]. This evidence is consistent

286 with results from dispersed oil enrichments yielding *Colwellia* spp. strain RC254, an isolate that could

287 degrade DOSS and oil in pure culture[39,68]. However, the initial microbial community influenced

288 enrichment outcomes. Mesocosms using surface water GOM inocula containing mostly *Vibrio* spp. did

289 not enrich *Colwellia* spp. with Corexit or dispersed oil[73]. Mesocosms in the same study using a deep GOM

290 water column community inoculum for Corexit 9500-only enrichments saw a marked increase in *Colwellia*

291 activity and an increase in *Winogradskyella* spp. abundance[73]. When similar experiments were repeated

292 with other inocula and alternative oil sources, *Marinobacter* spp., *Alcanivorax* spp., *Cycloclasticus* spp., and

293 *Alteromonas* spp. [72,77,78] were enriched in treatments with Corexit 9500 or dispersed oil. Regardless of the

294 specific organisms enriched, many of these mesocosm experiments showed distinct and repeatable

295 differences between microbial responses to oil and dispersed oil, suggesting phylogenetic or metabolic

296 differences manifesting between the two substrate mixtures.

297 Some incubation studies showed enhanced oil degradation[40,73] with Corexit while others observed no

298 enhancement[74,75] or suppressed[66] hydrocarbon biodegradation in the presence of Corexit (reviewed in

299 REF[79]). Where enhanced biodegradation occurred, degradation followed the typical pattern in which

300 lower molecular weight hydrocarbons, and those in more labile forms like alkanes, were preferentially

301 degraded relative to larger molecules or those with aromatic structures such as polycyclic aromatic

302 hydrocarbons (PAHs)[67]. Thus, while Corexit 9500 can sometimes improve biodegradation at low oil

303 concentrations and/or higher temperatures, it has a range of effects that include inhibition of

304 biodegradation, at least in limited laboratory studies. Such experiments are challenging to design and

305 interpret due to the uncertainties associated with complex initial microbial communities, disparate oil

306 sources, the physical form of the oil and dispersants, and the difficulty of mimicking partitioning and

307 dilution that occur in the environment. Nevertheless, the release of oil and dispersant chemicals applied

308 during the DWH disaster provided an opportunity to link basic microbial concepts with the real-world

309 complexity of marine ecosystems, providing insight to the interplay of petroleum's physical and chemical

310 behavior with seed populations, metabolic specificity, nutrient availability and growth dynamics, and

311 interaction with the non-microbial components of the deep-sea ecosystems.

312 **[H1] Oil weathering at the sea surface**

313 Oil is an exceedingly complex mixture, comprised of millions of compounds containing primarily carbon

314 and hydrogen, and sometimes oxygen, nitrogen and sulfur[1] (Figure 5). Each of these molecules has

315 different physical-chemical properties and reactivities towards light and biology, and on the ocean

316 surface, they are subjected to environmental weathering processes, including evaporation, dissolution,

317 biodegradation, and photo-oxidation[5]. Weathering physically fractionates the initial oil composition

318 between phases (such as oil residue, water, and air) and chemically alters the original oil molecules[1,80-83].

319 Evaporation from the sea surface removes low-boiling ("light") compounds, whereas dissolution

320 removes remaining 1-3 ring aromatics with few alkyl groups[81,84] and highly polar species, such as

321 naphthenic acids and alcohols[85]; however the extent of one process over the other depends on the the

322 physical-chemical properties of each compound. Prior to the DWH disaster, few studies (e.g., REF[86]) were

323 able to comprehensively apportion the relative importance of these processes due to under-sampling in

324 time and space, and analytical barriers to assess compositional differences between oil types[87]. The oil

325 released during the disaster was a light crude oil dominated by saturated hydrocarbons[1] **[G]** (~74%) and

326 as a result, up to 60% of its mass was lost to evaporation within hours to days of surfacing[81]. Due to the

327 subsurface oil release, though, dissolution played a larger role in the DWH disaster than in prior surface

328 spills where evaporation was dominant[88]. The physical changes and mass losses resulting from

329 evaporation and dissolution increases the viscosity of the remaining oil and the relative proportion of

330 surfactants present[89,90]. The enrichment of these naturally-occurring surfactants, and the physical mixing

331 of seawater into oil, can lead to emulsion formation. During the DWH disaster, thick emulsions were

332 formed from the oil that reached the sea surface, hampering oil recovery efforts and resisting most

333 microbial degradation[47,91,92].

334 However, the observed emulsions (also referred to as mousses) did not form solely from the result of oil-

335 derived surfactants or mass loss. They were also due to *in situ* photo-chemical changes, which modified

336 some oil components to products with more oxygen and increased surfactant-like properties (that is, they

337 were interfacially active and displayed both oil and water partial solubility)[93-96]. Specifically, photo-

338 oxidative weathering[97-99], the dominant weathering process acting on DWH surface oil[46,80], increased the

339 oxygen content of surface oil[80,83,100] and added ketone, hydroxyl, and carboxylic acid functionalized

340   (singly and in combination) hydrocarbons that were either lost to the air[36] or remained on the sea

341   surface[101]. The weathering changed the surface oil's physical properties, and reduced the effectiveness of

342   surface-applied dispersants to <45% at 2-4 days transit time on surface[91]. Further oxidation of

343   interfacially-active molecules yielded water soluble species that move from the oil into seawater as photo-

344   solubilized bioavailable carbon[102]. Indeed, as determined through ultrahigh resolution mass

345   spectrometry (Fourier-transform ion cyclotron resonance mass spectrometry; FT-ICR MS) , elemental

346   assignments for tens-of-thousands of molecules within each crude oil fraction (oil-soluble non-

347   interfacially active, oil-soluble interfacially active, and water soluble) indicate that (decreasing) carbon

348   and (increasing) oxygen number determined the progression of molecules from oil-soluble to water-

349   soluble[103]. Both the oil- and water-soluble photo-transformed species span aliphatic to highly aromatic

350   structures[80,101], indicating that these products originate from both aliphatic and aromatic hydrocarbon

351   precursors[104]. This suggests that both direct and indirect photo-oxidation contribute to the generation of

352   transformation products.

353   The use of novel analytical chemistry technologies developed in academic settings enabled detailed

354   quantitative estimation of photochemical oxygenation[100,104] and exploration of photochemical mechanisms

355   and rates[46,91,105] unattainable during previous oil disasters, such as the *Exxon Valdez* spill. In parallel,

356   advanced characterization of the molecules within the emulsions provided new insights into remediation

357   efforts aimed at these compound types[103]. Ultra-high resolution FT-ICR MS equipped with ionization

358   sources, such as electrospray-ionization (ESI) or atmospheric pressure photoionization (APPI) allowed

359   researchers to analyze oil (and oil weathering products) irrespective of boiling point. Thus, it provided

360   access to important molecular information on the changes induced by weathering for oil, interfacially

361   active species, and ultimately water soluble compounds that affect the fate, transport, toxicity, and

362   viscosity of surface oil[80,83,102,106,107].

363   Similarly, comprehensive two-dimensional gas chromatography (GCxGC) increased the chemical

364   resolution, assessment of chemical ordering, and capacity to model weathering processes achievable

365   through GC analyses, giving new understanding into source oil composition, biodegradation,

366   photochemical oxidation, water-column processes, and accurate measurements of biomarkers[104,108-

367   111]. These research efforts advanced the collective understanding of weathering and its effect on the initial

368   oil composition, which previously was characterized by conventional gas chromatography (GC)-based

369   techniques. The GCxGC approach is now scientifically mature and is used to examine changes in the

370   abundance of compounds and/or compound classes in the parent oil, which are then ascribed to various

371   categories of physical and chemical weathering[84,87,88,109,110,112-117]. However, even with the expanded

372   compositional information[104] through GCxGC, GC-based techniques are unable to detect many oxidative

373   transformation products, notably those that are highly oxidized with boiling points outside of the GC

374   range[118].

375   Although predicting the fate of discharged oil is extremely challenging on a molecular level and requires

376   knowledge of the original oil composition, new technologies facilitated substantial advances during the

377   DWH disaster. The complexity of oil requires a suite of analytical tools to comprehensively explore the

378   weathering mechanisms and products. In addition to the GC-based and FT-ICR MS tools highlighted

379   here, thin-layer chromatography flame-ionization detection (TLC-FID)[100] and Fourier transform infrared

380   spectroscopy (FT-IR)[119] provided quantitative and informative estimates of functional group changes

381   from weathering processes. Together, this suite of technologies provided a greater understanding of the

382   reaction mechanisms and impacts of weathering processes such as photo-oxidation and the identity of its

383   transformation products, as well as their impacts on physical properties, bioavailability and toxicity of

384   the discharged oil. This expanded understanding, combined with application of complementary

385   analytical platforms, will inform real-time responses in future oil spills.

386   **[H1] Broader environmental impacts**

387   The magnitude and duration of the Deepwater Horizon event, as well as its occurrence at depth, led to

388   impacts over a wide area that encompassed different types of ecosystems (**Fig. 6**). Coastal marshes and

389   beaches received oil washed ashore from the oil well discharge approximately 80 km away, while deep-

390   sea sediments[120] and corals[121] received fallout oil from the surface and intrusion layers in the form of oil-

391   associated detritus and mineral aggregates. Pelagic fish and cetaceans swam through surface and deep-

392   sea oil, and thus were exposed to the oil, gas and dispersant mixtures present in these areas[122-124]. While

393   the general impacts of oil on coastal organisms and environments were known prior to the DWH

394   disaster[125], the duration, magnitude, and scope of the discharge posed challenges to assessing the overall

395   impact and recovery, particularly in the deep-sea where baseline studies are rare to nonexistent. In many

396   cases, the ecosystems recovered as predicted from previous oil discharge studies, while in others, the oil

397   discharge combined with other stressors to cause larger impacts than expected[121,126,127].

398   Oil contaminated over 2000 km of Gulf Coast shoreline, half of which was located in Louisiana[8]. The

399   oiling was patchy in both quantity and location, and impacted predominantly marsh ecosystems[128] and

400   beaches[129]. For marshes, instances of heavy oiling (such as seen in Barataria Bay, LA) led to substantial

401 grass die-off at the marsh edge and subsequent enhanced shoreline erosion[127] led to permanent damage to

402 the marshes. In marshes that were less oiled, grass re-growth was evident after two years[128]. On oiled

403 beaches, oil-contaminated sands hosted blooms of hydrocarbon-degrading bacteria (such *Alcanivorax*, a

404 genus within the Oceanspirallales[130], and *Candidatus* Macondimonas diazotrophica[131]) within days of

405 oiling, followed by a succession to bacteria (such as *Rhodobacteriaceae*) that likely degraded more

406 recalcitrant oil compounds over the course of the disaster[130]. Despite the removal of large quantities of

407 oiled materials during the response phase[132], oiled sand patties were found years after the spill on

408 beaches in the states of Florida, Alabama, Mississippi and Louisiana[100,133]. Many of these oil-sand patties

409 are impervious to water and have few nutrients, thus they are not effectively degraded by bacteria[134].

410 However, other than being unsightly, no significant problems or concerns have been identified with these

411 oil residues, although for unknown reasons, the pathogen *Vibrio vulnificus* was enriched on these 'tar

412 balls' compared to sand and seawater[135]. Continued monitoring of the oil content in marshes and beaches

413 is necessary to understand long-term impacts and recovery of these ecosystems to pre-DWH conditions.

414 Compared to the relatively well-understood nearshore ecosystems, the impact of oil from DWH on the

415 deep-sea was unprecedented and unknown[136]. A unique aspect of the DWH disaster was the observation

416 of significant flocculated material in sediments near the site of the blowout. This material was determined

417 to be aggregates of phytoplankton detritus, mineral aggregates, and microbial extracellular polymeric

418 substances (EPS), commonly referred to as "marine snow". During the DWH disaster, marine snow

419 captured oil and then sank to the seafloor, transporting oil through the water column in a transport

420 process dubbed Marine Oil Snow Sedimentation and Flocculent Accumulation, or MOSSFA[137-139]. Surface

421 dispersant applications appear to enhance floc **[G]** formation in experimental systems[39,66,140,141] due to

422 interactions of EPS and dispersants that form quickly-sedimenting material[142]. MOSSFA-derived oil on

423 the seafloor is estimated to be between 0.5-14% of the total oil released[14,15], and two regions of the sea

424 floor with heavy marine oil snow suggest that the spatial extent of MOSSFA deposition is between 12,805

425 and 35,425 km^2 (REF[143]). Sediment microbial communities responded to the influx of hydrocarbons to the

426 seafloor, including the enrichment of *Colwellia* and uncultured gamma-proteobacterial strains that were

427 similar to strains observed in the subsurface intrusion layer[144]. In heavily-oiled sediments, genes from

428 anaerobic microbes such as *Desulfovibrionales, Desulfomonodales*, and *Desulfobacterales* in the

429 Deltaproteobacteria[145] and from anaerobic metabolism such as denitrification **[G]** [144] were observed. Even

430 after 4 years, significant oil residue was still present in sediments within 40km of the DWH blowout, with

431 biodegradation most inhibited in concentrated seafloor oil deposits[67].

432 Sedimented oil harmed deep-sea benthic organisms, causing decreases in sediment macro- and

433 meiofauna diversity[120,146] and high mortality rates of benthic foraminifera[147,148]. Coral communities were

434 also impacted, where octocorals were damaged or dead after being covered in flocculent material

435 containing oil and dispersant from the spill and response activities[136,149,150]. Recovery of the corals after

436 two years was mixed, with the average overall visible impact to corals on decreasing over time[151]. In some

437 instances, the tissue on the impacted corals had died and the remaining skeletons were colonized by

438 hydrozoa[151], indicative of coral deterioration. Colonization of corals by brittle stars, however, was a sign

439 of recovery as brittle stars are hypothesized to deter hydroid larvae from settling on coral branches[152]. As

440 recently as 2018, heavily oiled areas near the DWH blowout hosted lower biological diversity of deep-sea

441 megafauna, although with higher numbers of arthropods (such as crabs and shrimp) in many sites[153],

442 underscoring the long term impact of the DWH disaster on benthic communities.

443 **[H1] Outlook**

444 The needs and opportunities following the Deepwater Horizon disaster led to a renaissance in oil spill

445 science, with numerous breakthroughs on the transport and behavior, fate, and short- and long-term

446 effects of acute releases of oil and gas in the marine environment. Scientists and engineers from outside

447 oceanography fields were engaged; a generation of scientists was trained; new chemical and biological

448 methods were developed, often with novel technology or modified from other scientific fields; highly

449 detailed insights were gained on the rates, specificity, mechanisms, and products of abiotic and biotic

450 processes; and case studies, lessons learned, and recommendations on mechanical and chemical response

451 technologies were proposed and presented[27,154].

452 In the 10 years since the disaster, detailed monitoring and observations have yielded the most

453 comprehensive oceanographic investigation of the northern Gulf of Mexico. The collective scientific effort

454 identified what was unknown before the DWH (photo-oxidation of surface oil, MOSSFA, deep-sea

455 dispersant use and fate) and prioritized new knowledge that was necessary to change the scientific or

456 practical response to the spill. There is now a deeper understanding of the microbial degradation of

457 petroleum hydrocarbons and whether nutrient stimulants can or should be applied to offset potential

458 nutrient limitation during bioremediation (while being mindful to avoid full oxygen depletion, which

459 would shut down efficient aerobic degradation)[56]. There have been several paradigm shifts in our

460 understanding of oil weathering, most notably regarding the onset and rates of oil photo-oxidation[46], and

461 the molecular specificity of its transformation products. In particular, we learned that oxygenated

462   products from oil photo-oxidation are polyfunctional[101], impact the efficacy of surface applications of

463   dispersants[91], and have very complex roles in emulsion formation[103]. With the ability to isolate and

464   characterize these transformation products, new mitigation strategies can be developed.

465   How the results from the DWH disaster can be applied to future of oil spills must be considered, as

466   drilling and recovery technologies are implemented and deep-sea oil drilling is expanded, wider and

467   multi-seasonal shipping lanes in northern latitudes are opened, above and below ground pipelines near

468   aquatic environments are installed, and oil recovery in remote locations is increased[155]. The response and

469   continued studies of the DWH disaster benefited from the relatively accessible location for a deep-water

470   well (80 km offshore) and nearly-optimal weather (favorable summer currents, mild winds and calm

471   seas). Along the relatively populated Gulf Coast, responders and scientists used existing infrastructure

472   for shipping, housing, and land and water access. A more remote location with limited infrastructure,

473   such as in the high-latitudes would prohibit a commensurate response, and would effectively exclude

474   academic scientists. A further complication would be the uncertain impact of temperature and different

475   oil composition on the processes observed in the DWH disaster, such as microbial biodegradation, floc

476   formation, and weathering. For example, dispersants seem to be less effective at stimulating

477   biodegradation at colder temperatures[40,73]. In addition, heavier crude oils in high-latitude reservoirs are

478   more polar and contain more oxygen, nitrogen, and sulfur than those discharged during DWH;

479   consequently they will be more viscous, harder to disperse, and less bioavailable[156]. Lastly, some studies

480   have implicated the polar components of oil in higher ecotoxicity to resident fish populations[157], with

481   implications for local fisheries and tourism. All of these factors inhibit simple extension of DWH results

482   to a high-latitude spill.

483   A critical area for additional research lies at the nexus of systems biology and analytical chemistry in the

484   context of oil degradation. Metabolic databases are relatively complete for the degradation of simple

485   hydrocarbons and aromatic compounds like BTEX but they are missing information on the metabolic

486   pathways that degrade heteroatom-containing hydrocarbons[158]. In other words, we can detect

487   degradation products but do not know which genes or microbes are responsible for producing them from

488   the source oil. Moreover, as there could be substantial functional redundancy between different oil-

489   degrading communities[60,78,159-161], identifying the members of the microbial community may not matter in

490   assessing degradation potential if the functional genes are present and expressed similarly. Ideally,

491   chemical or biological markers will be identified that allow assessment of the "state" of an oil discharge

492   event, supporting response strategies that increase oil degradation rates. Inexpensive portable long-read

493   sequencing technology, like that from Oxford Nanopore, might provide near real-time microbial

494   functional gene expression measurements, enabling adaptive responses and mitigation strategies tuned

495   to microbial functionality[56]. As high-throughput approaches for reconstructing microbial genomes,

496   monitoring chemical transformations, and combining biological and chemical characterizations mature,

497   this problem is poised to be solved.

498   One of the biggest open questions after the DWH disaster concerns the use of dispersants and their

499   complicated cost-benefit calculations. A primary goal of dispersant application during DWH was

500   reduction in oil droplet size to enhance sequestration of oil in the deep sea[37]. The potential to enhance

501   biodegradation was a secondary consideration and although dispersants potentially improve

502   hydrocarbon degradation, they could also prevent or delay this desirable impact, depending on

503   environmental and biological circumstances[27]. Interdisciplinary teams of chemists, biologists, ecologists

504   and geochemists must work to ascertain the conditional tradeoffs in dispersant use, particularly in deep-

505   sea environments where degradation potential is greater but oxygen is finite and megafauna such as

506   pelagic fish and sessile corals have much longer generation times and slower recovery

507   times. Importantly, tradeoffs with dispersant use are not binary, adding another layer of complexity to

508   the decision process. For example, if the subsurface dispersant to oil ratio effectively modulates droplet

509   size, it is possible to envision a scenario in which dispersant applications could be used dynamically to

510   control the extent, rate and location of oil reaching the surface.

511   Between the *Exxon Valdez* and the DWH disaster, expertise in the academic community on oil spill

512   response and oil degradation dwindled due to lack of funding and the effectiveness of the Oil Pollution

513   Act of 1990 to assign blame and assess fines. Although the scientific community was able to re-tool

514   quickly to respond to DWH, we are now at a similar crossroads with the ending of the GoMRI research

515   program and the fading memory of the 2010 event. A substantial cohort of graduate students,

516   postdoctoral researchers, and early career scientists have been trained in oil chemistry and microbiology,

517   but their future interests, job prospects in this field, and funding portfolios for federal and private

518   agencies are uncertain. The DWH disaster benefited from the expertise of scientists and responders who

519   were involved in the *Exxon Valdez* and the *Ixtoc* spills (1979, in the Gulf of Mexico), but many of these key

520   knowledge-holders are retiring. Even if they are replaced, there will be a loss in critical mass of talented

521   individuals that can apply and interpret the new chemical and biological technologies. We are concerned

522   about future funding for known knowledge gaps, particularly in the usage of dispersants. A consortium

523   of academics, government scientists and industrial partners must continue to explore these chemicals and

524 their short- and long-term impacts on marine environments. While there have been great strides with

525 academia developing and collaborating with government and industry, these relationships should be

526 fostered and clear channels of communication between these different groups must be strengthened[162].

527 These relationships will allow for trust building and recognition that while the interests of academia are

528 not always lockstep with the response community, overlapping interests can provide key opportunities

529 for future funding and collaborative research[162].

530 As we look back on the past decade, a few signals rise from the collective noise and provide a framework

531 for planning effective responses to future oil spills. First, scientific findings and recommendations should

532 influence policy and regulators, with a strategic embrace of complex biological and chemical data as

533 diagnostics of environmental processes. Second, the intricacies of interacting microbes and environmental

534 factors make understanding the oceans before, during, and after oil spills extraordinarily challenging,

535 requiring a plan for interacting and complementary analytical platforms across molecular biology and

536 chemistry. We cannot retreat again to one or two analytical methods as they unintentionally blind us to

537 the interdependent cascade of environmental processes, some of which are likely to be more important in

538 the next spill than they were in the DWH disaster. Finally, response teams and research collaborations

539 with common purpose are capable of working together to understand the impact of this spill on affected

540 ecosystems such as the Gulf of Mexico; in so doing, they are responding to a critical societal need.

541

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Figures

Figure 1. (a) Map of oiled area between April and July 2010 with surface dispersant application, adapted from ERMA. Research expeditions to assess the extent of the subsurface intrusion occurred between May and July 2010, within the region denoted by the (color) circle. (b) The time-line of sampling from research vessels, aerial surveys and the IGT subsurface sampler. Adapted from REF[5].

Figure 2. Integration of technologies used in Deepwater Horizon oil spill response. Numerous oceanographic tools were deployed to assess surface and deep expressions of oil, gas and dispersants between April and July 2010. These included autonomous vehicles, water and sediment sampling equipment, and aerial assets. Adapted from REF[163].

Figure 3. Distribution of DWH hydrocarbons in three primary reservoirs in the Gulf of Mexico: the atmosphere (A), the ocean surface (B) and the subsurface intrusion (C). During the spill, oil and gas were partitioned among these reservoirs (D). Adapted from REF[5].

Figure 4. Schematic of subsurface intrusion, microbial succession and flocculent material. A typical depth profile of hydrocarbon concentrations near the MC-252 wellhead (left) shows the location and vertical extent of the subsurface intrusion. Microbes degraded oil and gas, with different microbes blooming at different times. Dispersants were present in both the subsurface intrusion and the surface slicks, while flocculent material (or MOSSFA) was deposited on the sediment floor.

998   **Figure 5.** Analysis of DWH oil and field samples (sand/oil patties collected from GoM beaches) with four
999   different techniques that captures collective effects of abiotic and biotic weathering, which are manifested
1000   in changes of: (**A**) GC-FID chromatograms, (**B**) TLC-FID chromatograms, (**C**) FT-IR absorbance, and (**D**)
1001   broadband FT-ICR-MS spectra. Overall, weathering lead to degradation of saturated and aromatic
1002   compounds leaving behind recalcitrant compounds in the unresolved complex mixtures, to an increase in
1003   OxHC fractions relative to saturated and aromatic compounds, and to increases in hydroxyl and
1004   carbonyls. The (-)ESI FT-ICR-MS revealed a complexity increase in the number of peaks (from m/z 200–
1005   1000) and the appearance of oxygenated species (red peaks) in a mass-scale expanded 400 mDA segment.
1006   Of note, similar molecular information is available for all other nominal masses in the mass spectrum.
1007   Panels A-C modified from REF[100]. Credit: C. Aeppli, C. Reddy, R. Rodgers

1008   **Figure 6.** Comparison of oiled (right) and non-oiled (left) marine ecosystems, during the DWH disaster.
1009   Primary impacts included the subsurface intrusion, oiled beaches and marshes, and oiled sediments and
1010   corals.

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1013 **Glossary terms**

1014 Amphiphilic: a chemical compound that has both hydrophilic and hydrophobic properties

1015 Denitrification: a multi-step microbial process that reduces nitrate to molecular nitrogen

1016 Dispersants: chemical mixtures used during oil spill response to break up and decrease the size of oil
1017 slicks or oil droplets so that they can be more easily mixed with water

1018 Floc: a mass of loosely-associated particles formed from the aggregation of minerals and organic particles
1019 suspended in water

1020 Metagenomics: the study of the genes (DNA) present in a mixed community, provide an assessment of
1021 metabolic potential in that community

1022 Metatranscriptomics: the study of the transcripts (RNA) present in a community, provides a snapshot of
1023 the genes being expressed at that time

1024 Photo-oxidation: a chemical modification reaction resulting from the absorption of light in the presence of
1025 oxygen

1026 Saturated hydrocarbons: chemical compounds that are comprised of carbon and hydrogen
1027 (hydrocarbons) in which all carbon-carbon bonds are single bonds.

1028 Stable isotope probing: a technique to trace the microbial consumption of a substrate through the
1029 examination of the stable isotopic composition of the substrate and the resulting biomass of the consumer