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## A review of the toxicology of oil in vertebrates: what we have learned following the *Deepwater Horizon* oil spill

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### ABSTRACT

In the wake of the *Deepwater Horizon* (DWH) oil spill, a number of government agencies, academic institutions, consultants, and nonprofit organizations conducted lab- and field-based research to understand the toxic effects of the oil. Lab testing was performed with a variety of fish, birds, turtles, and vertebrate cell lines (as well as invertebrates); field biologists conducted observations on fish, birds, turtles, and marine mammals; and epidemiologists carried out observational studies in humans. Eight years after the spill, scientists and resource managers held a workshop to summarize the similarities and differences in the effects of DWH oil on vertebrate taxa and to identify remaining gaps in our understanding of oil toxicity in wildlife and humans, building upon the cross-taxonomic synthesis initiated during the Natural Resource Damage Assessment. Across the studies, consistency was found in the types of toxic response observed in the different organisms. Impairment of stress responses and adrenal gland function, cardiotoxicity, immune system dysfunction, disruption of blood cells and their function, effects on locomotion, and oxidative damage were observed across taxa. This consistency suggests conservation in the mechanisms of action and disease pathogenesis. From a toxicological perspective, a logical progression of impacts was noted: from molecular and cellular effects that manifest as organ dysfunction, to systemic effects that compromise fitness, growth, reproductive potential, and survival. From a clinical perspective, adverse health effects from DWH oil spill exposure formed a suite of signs/symptomatic responses that at the highest doses/concentrations resulted in multi-organ system failure.

### KEYWORDS

Oil toxicity; *Deepwater Horizon* oil spill; wildlife toxicology; Gulf of Mexico; fish; birds; sea turtles; marine mammals; human health





























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**Table 1.** Summary of reported effects of DWH oil exposure on vertebrate systems, life stages, and responses. Effects are dependent on oil type, exposure concentration and duration, exposure route, and species/life-stage. Although the focus is mainly on toxicological effects, we include some physical effects of oil, particularly in birds, turtles, and humans. Workshop participants provided their expert opinions on relative weights of evidence for effects on a given system. Red symbols denote the taxa in which the most significant effects on the system have been reported or there is a large amount of evidence for effects on the system. Black symbols denote the taxa in which there is some limited evidence for an effect. Cells with no text indicate no data are available for that system/taxon combination. The studies included under the behavioral “system” are a collection of tested effects in whole animal “behaviors”, but the etiologies of the response impairments likely differ and mechanistically may belong to other physiological systems. Downward arrows represent a decrease in the observations/effects; upward arrows represent an increase. HPI: hypothalamus-pituitary-interrenal axis; HPA: hypothalamus-pituitary-adrenal axis; ROS: reactive oxygen species; LPO: lipid peroxidation.

Stage/System/ Response	Taxa				
	 Fish	 Birds	 Turtles	 Marine Mammals	 Humans
Development and early survival (Section 1)					
Reproduction (Section 2)	Deformities ↑ 				
	Possible transgenerational effects Reproductive success ↓	Reproductive success ↓ Parental care ↓ Nest abandonment Hatching success ↓	Variability in hatching success	Reproductive success ↓ Fetal distress	
Endocrine (Section 3)					
	Possible HPI dysfunction	HPA dysfunction	Possible HPA dysfunction	Impaired stress response – probable HPA dysfunction Adrenal gland disease	Impaired stress response
Respiratory (Section 4)					
	Gill damage DNA single strand breaks in gill. Observed asphyxiation Maximal metabolic rate ↓ Aerobic scope ↓	Cough/dyspnea Air sacculitis Open-mouthed breathing	Observed asphyxiation	Moderate-severe lung disease Bronchopneumonia	Respiratory disease
Cardiovascular (Section 5)					
	Cardiotoxicity in early life stages Disruption to K <sup>+</sup> and Ca <sup>2+</sup> ion fluxes Stroke volume ↓ Cardiac output ↓ Cardiomyocyte shortening during stimulation ↓	Damage to myocytes Myocardial contractility ↓ Arrhythmia Possible dilative cardiomyopathy	Possible effects indicated by changes in hematology and blood chemistry	Possible cardiac morphometric abnormalities and cardiac fibrosis	Heart attack risk ↑ Palpitations ↑ Acute chest pain
Neurological/ Sensory/ Behavioral (Section 6)					




(Continued)

**Table 1.** (Continued).

Stage/System/ Response	Taxa			
	Visual acuity ↓ Olfaction ↓ Critical swim speed ↓ Swim performance ↓ Prey capture ability ↓ Thigmotaxis ↑ Group cohesion ↓ Risk taking behavior ↑	Flight control ↓ Take off speed ↓ Flight length ↑ Foraging time ↓ Flight strategy change Lethargy Heat-seeking behavior		Mental health ↓ Dizziness, nausea, migraine ↑
Nutritional/ 	Gastrointestinal/Urinary (Section 7)			
	Altered gut microbiome	Food consumption changes Slower refueling post-exercise Hematochezia Renal ductular and tubular hypertrophy and mineralization Plasma urea and uric acid ↑ Kidney weight (normalized to body weight) ↑		Gastrointestinal symptoms ↑
Hepatobiliary (Section 8)				
	Hepatic lesions Biomarkers of hepatic activity ↑ Thermoregulation (Section 9)	Liver weight (normalized to body weight) ↑	Some indication of liver damage	
Integument/ 				
	Some evidence for skin lesion prevalence correlated to oil exposure	Hypothermia Feather damage ↑ Preening time ↑ Energetic flight costs ↑ Feather plucking ↑ Gross thickening of dermis	Hyperthermia when covered in oil	Limited in vivo evidence that PAH cause skin cell damage
Immune (Section 10)				
	Immune-associated gene expression ↓ Mortality following pathogen exposure ↑	White blood cell changes	T cell dysfunction	
Circulatory (Section 11)				
	Oxidative stress measured in circulating blood DNA single strand breaks in blood	Anemia coagulopathy		
Metabolism (Section 12)				

(Continued)

**Table 1.** (Continued).

Stage/System/ Response	Taxa		
	Growth rate over extended time ↓ Cellular damage (apoptosis, oxidatative stress) Cellular membrane lipid peroxidation from ROS (LPO measured in plasma) ↑	Foraging time ↓ Energetic flight costs ↑ Slower refueling Exercise-flight induced exhaustion Creatine kinase ↑ Aspartate aminotransferase ↑	Cellular damage oxidative stress ↑ Antioxidant responses ↑ DNA damage
Cellular Damage (multiple organ systems) (All sections)			
	Apoptosis, oxidatave stress ↑	Cytochrome P450 enzymes induced Glutathione and glutathione reductase induced	Oxidative stress ↑ Antioxidant responses ↑ DNA damage

## Introduction

Between April and July 2010, the Macondo well blowout/*Deepwater Horizon* (DWH) oil spill resulted in release of more than 636 million L of oil into the northern Gulf of Mexico (GoM). The resulting oil slicks covered over 112 million km<sup>2</sup> in the upper surface waters (Colwell 2014, 2018; DWH Natural Resource Damage Assessment Trustees 2016). Since the spill, a Natural Resource Damage Assessment (NRDA), along with a breadth of research projects funded by the GoM Research Initiative (GoMRI), have produced a large body of information on the exposure to and potential toxicity of DWH source and weathered crude oil and of the associated polycyclic aromatic hydrocarbons (PAHs) to marine vertebrates (DWH NRDA Trustees 2016). These include studies and reviews on fish (e.g., Pasparakis et al. 2019), birds (e.g., Bursian et al. 2017a; Dean and Bursian 2017), marine mammals (e.g., Takeshita et al. 2017), turtles (e.g., Mitchelmore, Bishop, and Collier 2017) and humans (e.g., Croisant and Sullivan 2018), but to date little effort has been put into surveying the effects of DWH oil across all of these vertebrate taxa.

GoMRI was established to investigate the impacts of oil, dispersed oil, and dispersant on GoM ecosystems. As part of this mission, GoMRI sponsored a workshop to discuss and synthesize the newly acquired knowledge on the toxicology of oil in vertebrates. The workshop, held in October

2018, included multi-disciplinary researchers from a broad array of study taxa. The workshop had the following specific goals –

- Collate and compare the results of exposure and effect (toxicity) studies at various marine trophic levels;
- Report commonalities and differences among effects on diverse species/life stages in laboratory and field studies; and
- Summarize the current state of knowledge following research arising from the response to the DWH oil spill.

Here we provide a review and summary of the workshop findings, along with additional relevant literature produced in the wake of the DWH oil spill.

A variety of DWH-related studies have documented impacts on survival, fecundity, and population dynamics in different species inhabiting the GoM that were within the footprint of the DWH oil spill (e.g., McCann et al. 2017; McDonald et al. 2017a, 2017b; Mullin et al. 2017; Schwacke et al. 2017; Wallace et al. 2017; Walter et al. 2014). However, the purpose of the workshop (and this subsequent review) was to focus on the toxicological aspects of DWH oil spill research in vertebrate species both during and after the event (i.e., investigations on the adverse physiological and pathological “sub-lethal” effects of the oil, rather than mortality and morbidity *per se*) (Table 1). The

**Table 2.** High level summary of toxicological study designs to describe the exposure and effects of DWH oil by taxon group. A major outcome of the workshop was that researchers and decision makers need to carefully consider the nature of each field/laboratory study's design, exposure/dose considerations, and context when comparing across experiments/field efforts and to estimates of exposure during the event (comparing these factors across the literature is beyond the scope of this review). Typically, health assessments were conducted and/or endpoints were measured on a similar time scale as the duration of exposure, however some tests investigated delayed exposures. This table only refers to studies cited in the body of this review; please refer to the citations in the text for more information about specific studies.

Taxon	Field or laboratory	Life stages	Exposure route(s)	Duration of exposure	Oil type	Concentration or dose known?	Other environmental factors	Internal measure of exposure?
Fish	Laboratory	Early life stages	Waterborne	Hours to days	WAF, HEWAF, CEWAF DWH slick oil, crude oil and PAHs	Water concentrations measured	UV and sunlight, temperature, salinity and dissolved oxygen	No
	Laboratory	Juvenile and adult Sub-adult adult Cells ( <i>in vitro</i> )	Waterborne and intraperitoneal Oiled sediment	Hours to weeks > month with 1 month recovery period	HEWAF, CEWAF, DWH slick oil, PAHs, DWH surrogate oil	Water concentrations measured Sediment and water concentrations for PAH monitored	Hypoxia Pathogenic bacteria	No Yes
	Field	Adult	Waterborne	Hours	WAF, DWH source and slick oil, DWH slick oil	Water concentrations measured		NA
Birds	Laboratory	Adults	Ingestion and "dermal", plus incidental dermal for ingestion exposures	Months to multi-year Days	DWH slick oil	Water concentrations measured		No
	Field	Adults	Ingestion and "dermal" (inferred)	Months to multi-year	DWH weathered oil	Yes, higher than turtle exposures		No
Turtles	Laboratory (surrogate turtle species)	Juveniles	Ingestion	Days to weeks	DWH slick oil	Yes, lower than bird exposures		Yes
	Laboratory (loggerhead hatchlings)	Hatchlings	WAF/CEWAF	Days		No		No
Marine Mammals	Laboratory	Juveniles and adults Cells ( <i>in vitro</i> )	Ingestion and dermal, plus inhalation.	Months	DWH oil	Estimated using qualitative scale, then extrapolated to quantitative ranges		Yes (for some studies)
	Laboratory	Adults	Waterborne	Weeks	WAF, CEWAF	Yes		N/A
Humans	Field	All life stages	Inhalation, dermal, ingestion, aspiration (inferred)	Years	DWH oil	No		No
	Field	Adults	Inhalation, some dermal and possible ingestion (inferred)	Months	DWH oil	Yes, estimated for inhalation		No



DWH oil spill resulted in a wide range of vertebrate species being exposed to the same source oil, albeit at different concentrations, stages of weathering, and exposure durations.

Cross taxa comparisons are perhaps more relevant than comparisons across studies with different oil types and sources, although there are efforts to standardize experiments across the fields (e.g., King, Elliott, and Williams 2020; Hodson et al. 2019; Adams et al. 2017; Mitchelmore et al. 2020; Engel et al. 2017). However, workshop participants repeatedly emphasized the need to appropriately contextualize the comparability of data and the limitations of the various approaches/methods, especially in relation to exposure and dose across the studies (Table 2). For some species such as sea turtles, birds, and cetaceans, information from earlier studies/reviews is included to provide connections about specific endpoints/systems and oil toxicity. Throughout the discussion, oil exposure refers to whole DWH oil, unless specifically stated otherwise (for example, PAH-only lab exposures). Although oil spill response actions can inadvertently result in toxicological impacts or complications, discussion of the combined impacts of oil and dispersant is limited here, as in the case of exposures to dispersed oil. However, robust literature on the effects of COREXIT on organisms is available (e.g., Anderson et al. 2011). To maintain the focus of the review, investigations concentrated on PAH-based toxicity. The specific toxicology of other chemical contaminants resulting from the DWH spill, such as heavy metals, was excluded but workshop participants acknowledged that these effects cannot be isolated in situations with whole oil exposures. However, the physical effects of oil, such as fouling of bird feathers, typically in the context of juvenile/adult birds and turtles. Where relevant, studies that include investigating the effects of multiple environmental stressors in addition to oil, such as the effects of temperature and sunlight, were included.

There are limitations to the comparisons that can be made across taxa in an effort to identify commonalities, pathways, target organs and toxicological endpoints. Clearly there are differences that are attributed to ecological niches, trophic levels, habitats, and life-history strategies of the different genera, as well as critical physiological differences. However, there are also limitations

due to the cryptic nature of marine species, challenges with regard to the study of long-term effects, and absence of baseline health data, in addition to ethical, logistical and funding constraints that have governed the studies. For example, investigations on the potential effects of DWH oil on endangered sea turtles have been limited to lab exposures with either un-releasable sea turtle hatchlings (Harms et al. 2019) or surrogate species such as red-eared sliders *Trachemys scripta elegans* and snapping turtles *Chelydra serpentina* (Mitchelmore et al. 2015). For lab tests, the study designs and methodologies, including the type of oil, concentration, duration of exposure, choice of native vs surrogate species, life stage, exposure route, and reporting of analytical chemistry, might also make cross-study comparisons difficult, especially when attempting to compare the findings to field studies.

The evidence for physical and toxicological effects are organized by target system and this review follows this organization (Table 1). The assembled scientists used their expert judgment to provide an indicative level of confidence and consensus regarding the degree to which the scientific evidence supported a conclusion that DWH oil affected a particular system and taxonomic group (see Table 1) and developed conceptual models/impact pathways as a means of synthesizing the information (Figures S1-S4).

### Exposure of vertebrates to DWH oil

The different types, magnitudes, durations, and combinations of vertebrate exposure to DWH oil, in addition to any other physical, biological, and chemical stressors, were likely to affect the comparisons of toxicity across studies and taxa (Table 2). However, as the main objective was to investigate common mechanisms, effects, and outcomes of oil toxicity, the group of experts agreed to avoid letting the overwhelming details of exposure derail the discussions. Even with the breadth and depth of the research associated with DWH, the question of how exposure influences toxicological outcome is a major gap in our collective understanding of oil's impacts on wildlife and humans due to the complex nature of the 1) duration and the



geographic scale of the spill, 2) variety of habitats, species, and life-stages affected, and 3) increasing number of sub-lethal oil effects at the different levels of biological organization. Thus, the variety of DWH oil toxicity exposure studies reflect the multi-faceted fate and transport of the DWH oil.

As source oil ejected into the GoM from the riser at the sea floor, it immediately mixed with sea water (and dispersants, once subsea application was in place) to produce droplets and dissolved oil components. Plumes of this DWH oil mixture then moved with the ocean currents. Similar mixtures of dissolved oil and oil droplets formed at the surface underneath the oil slick, driven partially by wave action and aerial application of dispersants. Many of the toxicological studies focused on the effects of waterborne oil on plankton, invertebrates, and fish, particularly focusing on: 1) negatively-buoyant early life-stage invertebrates and fish in the surface mixing zone, and 2) the likely sublethal effects to juvenile and adult fish, including an assessment of how exposure to oil droplets may differ from exposure to dissolved oil components alone (e.g., Morris, Lay, and Forth 2015). These lab experiments generally used precisely controlled conditions and concentrations as verified by analytical chemistry of water-accommodated fractions (WAFs) of oil using either high-energy mixing (HEWAFs) with a blender or stir-plate or chemically assisted mixing (CEWAFs) using dispersants.

Over time, waterborne oil components settled to the deep-sea floor, often due to the formation of oil and bacteria/particulate matter aggregates termed marine snow. As the remaining surface oil moved closer to coastlines, natural mixing and tidal action drove oil into shallow water sediment. Several groups conducted a variety of tests on benthic infauna, shrimp, and fish to understand how concentrating oil on the sea floor may impact fauna, including sub-lethal effects on older life-stages (e.g., Brown-Peterson et al. 2017, 2015a; Lotufo et al. 2016; Morris, Lay, and Forth 2015). Similar to the water accommodated fractions, conditions of these exposures may typically be controlled in the lab and measured using analytical chemistry including standardized mixing protocols to infuse sediment with various concentrations of oil.

At the sea surface, rising oil created an extensive surface slick and subsurface plume that, upon land-fall, covered the coastline with oil. In addition to embryonic and larval invertebrates and fish coming into contact with the slick and sheen, sea turtles and marine mammals encountered surface oil when surfacing to breathe, feed, and/or rest. Birds were also exposed to surface oil when landing on the water/coastline or foraging for prey, resulting in oily plumage. Thus, many lab studies exposed animals to naturally weathered slick oils (e.g., “slick A”) skimmed from the ocean surface during DWH response activities (see Forth et al. 2017 for a description and comparison of the oils used in the NRDA toxicity testing program). Several research groups specifically measured the effects of surface sheen on ichthyoplankton, including the additional negative effects of ultraviolet [UV] light. Other groups investigated exposure to other taxa, including a surrogate-species study on whole oil ingestion via esophageal gavage tubes with snapping turtles and red-eared sliders, and studies on the negative effects of DWH oil on bird species found in the GoM due to ingestion of oil-contaminated prey as well as oiled plumage. Although these experiments had similar delivery mechanisms, the types of oils were different (for example, natural vs. artificial weathering) and the final total dose was much higher in the oil-contaminated prey for birds compared to the turtle gavage protocol, thus these doses can only be compared to inferred oil exposures in the field. A summary of the different approaches to determine the impact of the oil on the major taxonomic groups is presented in Table 2. During the workshop, lab results were also compared to data on living and dead, wild animals collected during field wildlife response efforts for sea turtles (Stacy et al. 2017) and birds (Harr et al. 2017b; Wallace et al. 2017), and health assessments of temporarily captured common bottlenose dolphins (*Tursiops truncatus truncatus*) (Schwacke et al. 2017; Smith et al. 2017), as well as information from stranded birds (Fallon et al. 2018), turtles (Stacy et al. 2017), and dolphins (Venn-Watson et al. 2015). These investigations relied upon extrapolated and inferred exposures based upon field measurements or remote sensing data. However, many of the lab studies employed field-collected data about oil weathering and concentration to design and/or ground-truth/verify lab

exposure tests and results. Human studies included in our discussions relied upon reconstructed exposures to crude oil and dispersed oil (e.g., Stewart et al. 2018) and self-reports (Rusiecki et al. 2018).

Despite the variety of species, exposure scenarios, and additional stressors, a noteworthy set of consistent toxic effects was found for DWH oil across the literature likely driven in part by: 1) focusing on studies with one crude oil source, albeit at various stages of weathering, 2) using standardized methods for mixing oil and water or sediment, including analytical chemistry characterization and conducting lab exposures time, duration and concentration and 3) investigating a multitude of biological endpoints at various levels of organization from numerous sublethal effects to mortality on various biological systems.

### Systemic and physiological effects of oil on vertebrates

The workshop considered the evidence for toxicological impacts of DWH oil by broad physiological system; any affected systems and responses where findings were reported across more than one taxon are summarized in Table 1. There was also evidence for DWH oil's adverse effects on behavioral responses, life stage impacts, and on effects at the molecular and cellular level that may be common across different physiological systems. Where data were available by vertebrate taxonomic group, the findings of the studies are considered for each taxon fish, birds, turtles, marine mammals, and humans. Here, our conversations and available literature are summarized in the order of consideration at the workshop.

#### Development and early survival

Early life stages and juveniles across taxa may be more susceptible to the effects of oil due to their immaturity and developing physiologies.

#### Fish

*Pre-hatch.* The buoyant eggs of many fish species are likely to be at risk from the influence of oil exposure due to their distribution in the water

column, but their resilience may vary (Philiber et al. 2019; Wobus et al. 2015), particularly between saltwater and freshwater species. In combination with oil exposure, UV radiation from sunlight exhibits synergistic ability to enhance the effects of PAHs, known as photoinduced toxicity (Bridges et al. 2018), which is a particular risk for transparent eggs. Experiments with mahi-mahi (*Coryphaena hippurus*) embryos, both early (Alloy et al. 2016) and later in development (Sweet et al. 2017), determined that exposing them to WAFs of slick oil collected during the DWH spill, together with increasing levels of natural sunlight, significantly reduced the proportion that hatched and altered embryo buoyancy and metabolic rates. However, there may be different effects due to any discrepancies between UV spectra of natural sunlight versus artificial UV exposure systems (Lay et al. 2015). A similar effect on larval mortality was also detected in red drum (*Sciaenops ocellatus*), an economically and ecologically important estuarine species, and spotted seatrout (*Cynoscion nebulosus*) (Alloy et al. 2017), with the latter species being more sensitive. These findings highlight the importance of assessing the concentrations of PAHs associated with toxicity on fish embryos in relation to levels of UV (Lay et al. 2015). Evidence of interactive effects between temperature, salinity and PAHs on both hatching and embryo mortality in Gulf killifish (*Fundulus grandis*) was reported by Rodgers et al. (2018a). However, effects were not consistent and depended upon relative salinity and temperature combinations.

The type of oil is also a key toxicity factor as weathering of oil results in products with different compositions (Forth et al. 2017; Morris, Lay, and Forth 2015). Weathered oil was more lethal to mahi-mahi embryos, on a total PAH basis, than source oil based on 96 hr median lethal concentrations (45.8  $\mu\text{g/L}$  compared to 8.8  $\mu\text{g/L}$  total PAH) (Esbaugh et al. 2016). These differences may be related to the amount of dissolved  $\geq$  three-ringed PAHs in the oil although source and weathered oil initiated similar  $\text{LC}_{50\text{s}}$  in red drum (Khursigara et al. 2017). A difference between weathered oil and source oil was also found in bay anchovies (*Anchoa mitchillis*) in which the  $\text{LC}_{50\text{s}}$  were lower in embryos exposed to more weathered fractions of DWH oil

(O'Shaughnessy et al. 2018). Despite differences in weathering, concentration often remains the critical predictor of effects. For example, a genome-wide gene expression study in Gulf killifish determined that transcriptional responses only at higher concentrations of oil exposure were predictive of field-observed responses in the sensitive early life stages in addition to causing DNA damage in mature fish (Pilcher et al. 2014). Exposure of Gulf killifish embryos (< 24 hr post-fertilization) to HEWAF resulted in mortality that was exacerbated by simultaneous hypoxia or increased temperature (Rodgers et al. 2018a).

**Post-hatch.** Once eggs have hatched, the survival of the larvae is dependent upon the specific developmental window during which the larvae are exposed to oil. For example, Bay anchovies exposed to HEWAF and CEWAF displayed different sensitivities at two developmental stages. Young larvae at 5 days post hatching were more sensitive than older larvae at 21 days post hatching (Duffy et al. 2016). However, Duffy et al. (2016) concluded that lethal concentrations in acute (24 hr) exposure experiments were not sufficient for predicting effects in the wild or among species. Similarly, Brewton, Fulford, and Griffitt (2013) observed that DWH oil produced reduced growth in young spotted seatrout, but CEWAF initiated more adverse effects in larvae while HEWAF resulted in greater number of adverse effects in juveniles. Exposure to oiled sediment produced developmental malformations including yolk sac and pericardial edema, spinal defects, and tissue degeneration in zebrafish embryos (Raimondo et al. 2014). Gulf killifish larvae (< 24 hr post-hatch) exposed to HEWAF for 48 hr displayed decreased survival and development was negatively affected, but hypoxia and high temperature each enhanced adverse effects of HEWAF on development and mortality (Serafin et al. 2019).

Oil in sediment may also be toxic to epibenthic juvenile fish. In southern flounder (*Paralichthys lethostigma*) experimentally exposed to oil-contaminated sediments from Louisiana, mortality rates of juveniles were increased with both concentration and duration of exposure (Brown-Peterson et al. 2017) and gill abnormalities and hepatic lesions were observed in fish exposed to the most contaminated sediments.

## Turtles

Field and lab studies demonstrated no marked impact on mortality and deformity rates after turtle egg exposure to oil. After the 1979 Ixtoc 1 blowout in the Bay of Campeche, Mexico, lab studies with loggerhead turtle (*Caretta caretta*) eggs and field studies of Kemp's Ridley turtle eggs (*Lepidochelys kempi*) indicated that exposure to oil (not from DWH) reduced embryo and hatchling survival and produced developmental deformities in those that survived. Exposure conditions were important in determining severity of the effect: non-weathered oil was more toxic (Fritts and McGehee 1982). DWH oil exposure in surrogate sea turtle species such as common snapping turtles also diminished hatching success and increased developmental deformities (Bell, Spotila, and Congdon 2006; Van Meter, Spotila, and Avery 2006), although these were not seen by Rowe, Mitchelmore, and Baker (2009). In studies using non-DWH oil, altering the physical characteristics of the nesting substrate and/or physical smothering of the egg surface can impact eggshell respiratory function and hence embryo survival depending upon the particular part and proportion of the egg becoming fouled (Phillott and Parmenter 2001).

## Reproductive system

Many aspects of the reproductive system are susceptible to the toxic effects of contaminants, including the developing gonads, embryos, or through hormonal disruption. In addition, these outcomes affect the survival of the offspring with additional potential for long-term effects where contaminants are teratogenic. Due to the complexity of this system and various stages at which effects and impacts might be observed, a conceptual model was produced (Supplementary Information, Figure S1), illustrating the various stages of the reproductive cycle for different taxonomic groups, where effects were reported (details of the model are provided in the figure legend).

## Fish

**Egg production.** An investigation to determine the combined effects of chronic hypoxia and dissolved PAHs on egg production in sheepshead minnow (*Cyprinodon variegatus*) found

significant reduction under all treatments (Hedgpeth and Griffitt 2016), but the greatest decrease was noted when fish were exposed to hypoxic conditions in combination with a blended oil and water mixture and an oil, water, and chemical dispersant mixture. However, significant reductions were only detected when hypoxia was included as a factor, suggesting again that other stressors and prevailing environmental conditions such as dissolved oxygen need to be considered.

**Reproductive capacity.** Reproductive effects of oil on female spotted seatrout captured from Mississippi coastal waters following the DWH oil spill were assessed using a gonadosomatic index (GSI) and investigating ovarian development and spawning frequency (Brown-Peterson et al. 2015b). Overall, post-DWH spill fish displayed lower GSI values than those collected prior to the oil spill, particularly at the beginning of the reproductive season. The most striking finding was the effect on spawning frequency, which was markedly reduced post-spill, from approximately every 4.5 days to 13.5 days. Carr et al. (2018) examined the gonads of Gulf killifish one year after the DWH spill trapped at two impacted regions in Barataria Bay and one unimpacted site in East Texas. The GSI in males from the unimpacted area was twice that of males from the impacted site while testicular germinal epithelium thickness was 2.7-fold smaller at the impacted site. Carr et al. (2018) suggested that exposure to oil and dispersants may therefore adversely affect testicular function in these fish. Experimental exposure of sheepshead minnows to HEWAF under different oxygen and salinity conditions demonstrated that HEWAF exposure significantly lowered egg production and egg fertilization rate, but only under hypoxic and hypoxic with low salinity scenarios, suggesting that environmental conditions might directly modulate reproductive toxicity of oil exposure in fish (Jasperse et al. 2019a).

**Generational effects.** Transgenerational effects were reported in experimentally oil-exposed sheepshead minnows (Jasperse et al. 2019b). Two generations of offspring from fish dosed with HEWAF were examined. The  $F_0$  generation exhibited altered egg production and decreased

fertilization. Several developmental endpoints in the  $F_1$  generation were altered including reduced weight and length. Both exposed generations displayed deficits in prey capture compared to control  $F_1$  and  $F_2$  generations and correlations with tissue levels of PAHs provided evidence that these effects were associated with PAH exposure. A similar study design demonstrated that environmental conditions such as hypoxia and low salinity might enhance developmental abnormalities in unexposed sheepshead minnows born from parents experimentally exposed to HEWAF (Jasperse et al. 2019c). Bautista and Burggren (2019) reported that with adult zebrafish exposed to DWH oil through their diet,  $F_1$  larvae showed both beneficial and maladaptive traits, likely attributed to epigenetic transgenerational inheritance.

### **Birds**

**Reproductive capacity.** Prior to the DWH oil spill, a variety of studies established that oil exposure affects reproductive capacity in birds, by reducing mating behaviors, reproductive success, and parental care, as well as increasing nest abandonment (Barros, Alvarez, and Velando 2014; Fernie et al. 2018; Golet et al. 2002; King, Elliott, and Williams 2020; Leighton 1993; Walton et al. 1997). While experimental investigations of reproductive success such as egg laying, hatching success, deformity estimates, and fledging success were not conducted immediately following the DWH oil spill, Burger (2018) conducted population- and colony-based estimates of reproductive success (Burger 2018). Nesting phenology differed by oiling category for brown pelican (*Pelecanus occidentalis*), snowy egret (*Egretta thula*), tricolored heron (*Egretta tricolor*), laughing gull (*Leucophaeus atricilla*), and black skimmer (*Rynchops niger*), with the variation related to proportions of birds breeding earlier or later than peak nesting time. Burger (2018) suggested that there was no apparent evidence that reproductive success to fledging was affected in the year following the spill with the caveat that multi-year follow up may provide data on the long-term consequences of chronic oil exposure.



From 2008 to 2010, brown pelican nesting success varied across years, but was lowest in 2010, corresponding to higher habitat losses following the spill (Walter et al. 2013). Brown pelicans can nest on open ground; however, they prefer elevated platform nests in shrubs. Walter et al. (2013) observed that nesting success was positively correlated to nest height, and in some cases was negatively associated with nest density. In 2010, all 52 nesting attempts on Wine Island (Louisiana) were relegated to ground level and resulted in total abandonment prior to hatching (Walter et al. 2013). Reproductive failure due to habitat loss may result in adaptation to new nesting locations and partially account for lack of evidence for reproductive losses in colonial nesters in 2011.

### **Marine Mammals**

**Reproductive capacity.** Much of the information about the reproductive effects of oil in viviparous vertebrates comes from field studies of marine mammals. Lane et al. (2015) reported on the early follow up of common bottlenose dolphins exposed to DWH oil that were temporarily captured for health assessments. Only 2 of the 10 pregnant females produced viable calves compared to a previously reported success rate of 83% (Wells et al. 2014). Kellar et al. (2017) continued the investigation of reproductive success in two northern GoM bottlenose dolphin stocks and again found significantly lower rates of success when compared to unexposed reference stocks. Rates for both GoM stocks were 19.4% compared with 64.7% for control stocks. In addition, in the years immediately following the DWH oil spill there were elevated numbers of dead stranded perinatal bottlenose dolphins in the oil spill footprint and evidence of a higher prevalence of fetal lesions, including fetal distress (87 vs 27%), when compared to stranded perinatal dolphins examined from unexposed regions (Colegrove et al. 2016).

### **Turtles**

**Reproductive capacity.** Sea turtles are vulnerable to oil and associated oil spill response activities for a number of reasons, including direct physical fouling and toxicity of oil and indirect impacts to their

habitat (such as nesting beaches) or prey base (reviewed in Wallace et al. 2020). Loggerhead turtle nest densities on three NW Florida beaches in 2010 were reduced by 43.7% (95% CI: 10-65%) relative to expected nesting rates in the absence of a spill and its associated clean-up (Lauritsen et al. 2017). These reductions were attributed to both direct (e.g., mortality from oil and response activities) and indirect (e.g., deterrence of nesting) effects related to the DWH incident, particularly the high level of disturbance in the nesting beach areas resulting from response activities preventing females from coming ashore (Lauritsen et al. 2017).

### **Endocrine system**

The endocrine system is an important target system for many contaminants, including PAHs, as many are known to be endocrine disruptors, often mimicking the effects of hormones through receptor binding (Lee et al. 2017). The hypothalamus-pituitary-inter-renal (HPI) axis is an interacting regulatory system governing the release of cortisol that is critical in how fish respond to many environmental stressors and social structure. The HPI axis is analogous to the hypothalamus-pituitary-adrenal (HPA) axis found in mammals, birds, and turtles, which also serves as an important regulator of stress response and a modulator of neuroendocrine-immune interactions (Parent et al. 2011). A conceptual model of the endocrine system in the context of this review and potential effects of oil is illustrated in Supplementary Information, Figure S2.

### **Fish**

**Hypothalamus-pituitary-inter-renal axis.** Khursigara, Ackerly, and Esbaugh (2019) reviewed evidence for interactions between oil exposure and environmental stressors such as hypoxia, temperature, salinity and acid-base balance in fish through the HPI. Findings suggest that oil exposure puts fish at a competitive disadvantage, although the mechanism through which this is acting is not clear. Reddam et al. (2017) also indicated that the glucocorticoid stress response is stimulated in toadfish (*Opsanus beta*) exposed to PAHs. Evidence from *in vivo* and *in vitro* experiments indicated that there may be internalization or

downregulation of the melanocortin 2 receptor in response to PAH exposure. This receptor mediates the action of adrenocorticotrophic hormone (ACTH) and exposed fish were no longer able to mount a second response when subjected to stressful conditions associated with crowding.

### **Birds**

**Glucocorticoids.** A case study of northern gannets (*Morus bassanus*) breeding in Eastern Canada investigated the impact of PAHs on circulating prolactin and corticosterone, two hormones that are involved in metabolism and mediation of the stress response and influence reproduction. Franci et al. (2014) reported that 23.5% of the birds had overwintered in the GoM in 2010-2011 during the time of the DWH oil spill, but found no apparent evidence for effects of oil on hormones or body mass. However, studies on house sparrows (*Passer domesticus*) demonstrated an impaired stress response after ingestion of DWH oil (Lattin et al. 2014). The sparrows' ability to elevate corticosterone in response to both a standardized stressor and an injection of ACTH was impaired following 4 weeks of exposure to a 1% crude oil diet. In addition, a second house sparrow study demonstrated tissue-specific changes in the density of glucocorticoid receptors following 6 weeks of ingestion exposure to 1% weathered crude oil, including lower receptor numbers in liver, high numbers in fat, and no marked changes in kidneys, muscle, spleen, or testes (Lattin and Romero 2014)

Feather corticosterone and nestling body condition were measured in 3- to 4-week-old brown pelicans nestlings along the GoM from 2013 to 2015 (Lamb, O'Reilly, and Jodice 2016). Feather corticosterone may be employed an indicator of chronic stressors, and in the case of nestling or fledging birds, chronic elevated stress might impede development. While the study did not directly assess the impact of oil exposure on nestling health, data demonstrated a negative correlation between body condition and increased feather corticosterone such that nestlings from ground or lower level nests exhibited higher feather corticosterone and lower body condition. As discussed in the section on bird reproduction, brown pelicans in some areas along the GoM lost optimal nesting sites producing reproductive failure in 2010 (Walter et

al. 2013). However, Lamb, O'Reilly, and Jodice (2016) indicated that chronic stress of this habitat destruction has been impacting nestling success in subsequent years.

### **Marine Mammals**

**Glucocorticoids.** One of the most striking findings following the DWH spill was its impact on adrenal function in bottlenose dolphins (Schwacke et al. 2014). Dolphins temporarily captured in an oil-impacted area (Barataria Bay, Louisiana) displayed exceptionally low serum concentrations of two key adrenal hormones, cortisol and aldosterone, when compared to concentrations found from prior dolphin health assessment studies across multiple sites (Schwacke et al. 2014). Many of these animals were not able to mount an expected acute stress response following the capture event, which should include elevated serum cortisol and potentially aldosterone levels. These findings were reinforced by the pathology results among the dolphins found dead following the spill (Venn-Watson et al. 2015). Adrenal tissue examined histologically showed that a high proportion (33%) exhibited thin adrenal cortices, compared to 7% in dolphins stranding outside the area affected by the spill. A follow-up study carried out 3–4 years post-spill demonstrated that impaired stress response persisted for at least 4 years after the disaster (Smith et al. 2017).

### **Turtles**

**Hypothalamic-pituitary-adrenal axis (HPA).** No marked differences in baseline corticosterone concentrations were found in either surrogate sea turtle species (red-eared sliders and snapping turtles) exposed via gavage for 14 days to DWH oil collected from the ocean surface ("slick A") at 100 and 1,000 mg/kg (Mitchelmore et al. 2015). Alterations in concentrations of stress-induced corticosterone via ACTH with increasing oil dose were not observed, although a non-significant trend to lower levels was detected in red-eared sliders (Mitchelmore et al. 2015). However, two red-eared sliders in the 1,000 mg/kg treatment failed to respond to clinically normal corticosterone concentrations such that the potential for an impact to the HPA axis cannot be ruled out, especially given the limited number of individuals, relatively short exposure time of 14 days (compared to weeks and

months for studies in other reptiles and mammals), and inability to follow any delayed impacts (Mitchelmore et al. 2015).

### **Respiratory system**

The respiratory system is susceptible to acute and chronic toxic effects of airborne pollutants, including the vapor phase of crude oil components, particulate PAHs, and volatile organic compounds (VOCs) that are produced by large crude oil slicks. A conceptual model of the respiratory system and DWH oil effects by taxa is illustrated in Supplementary Information, Figure S3.

#### **Fish**

**Respiratory function.** While the evidence that acute oil exposure can impair cardiorespiratory function in larval fish is robust (Mager et al. 2014), the effects on later life stages were less well known before the DWH oil spill. Young fish (mahi-mahi, Atlantic croaker [*Micropogonias undulatus*], and red drum) experimentally exposed to DWH oil at environmentally relevant concentrations exhibited reduced maximal oxygen uptake, lower aerobic scope, and decreased swimming capacity (Johansen and Esbaugh 2017). Swim performance was affected at a lower PAH concentration whereas a 3-fold rise in exposure from 4 to 12  $\mu\text{g/L}$  total PAH<sub>50</sub> affected both movement and respiration.

**Low oxygen tolerance.** Hypoxia tolerance, considered here as the critical oxygen threshold, was investigated in Atlantic croaker experimentally exposed to non-weathered crude oil (Pan et al. 2018). Fish exposed to 10 and 23  $\mu\text{g/L}$  total PAH showed a significantly reduced aerobic scope relative to controls. This was driven exclusively by diminished maximal metabolic rate with no marked effect on standard metabolic rate. In red drum, hypoxic conditions and oil exposure resulted in additive reductions in aerobic scope compared to either stressor alone (Ackerly and Esbaugh 2020). Zhang et al. (2017) also suggested that this may not be consistent among individuals, with hypoxia tolerant individuals being more susceptible. Individual European sea bass (*Dicentrarchus labrax*) subjected to a hypoxia challenge test responded differently. Interestingly, the hypoxia

tolerant phenotypes remained chronically impaired following a 24 hr oil exposure bout. Zhang et al. (2017) concluded that this was due to inferior hypoxia resistance through the glycolytic metabolic pathway at the oiled gill, in hypoxia tolerant fish.

#### **Birds**

In western sandpipers (*Calidris mauri*) orally dosed with artificially weathered DWH oil at concentrations of 1 or 5 ml kg/body weight/day for 20 days, there was presence of pulmonary hemorrhage in the treated groups but not control, indicating damage to the lungs and a decreased ability to exchange oxygen (Bursian et al. 2017b). Dermally treated double crested cormorants (*Phalacrocorax auritus*) were noted to display significant dyspnea upon handling and examination. Control birds examined within 24 hr exhibited no evidence of respiratory distress. Further, bradycardia likely induced by the dive reflex was noted in treated cormorants only. Histologically, hemorrhage (likely due to euthanasia) and inflammation were also present in wild-caught control groups; thus, respiratory lesions could not be assessed in this study due to a low sample number (Harr et al. 2017b).

Chicken hatchlings exposed *in ovo* to DWH HEWAF airborne volatiles showed changes in *cyp1a* expression in lung epithelia that were indicative of PAH exposure (Dubansky et al. 2018). These changes in *cyp1a* might potentially result in similar effects on heart and lung function following *in ovo* exposure to dioxins or PCBs (DeWitt et al. 2006; Kopf and Walker 2009).

#### **Marine Mammals**

**Respiratory system abnormalities.** Schwacke et al. (2014) found a significantly higher proportion of the bottlenose dolphins in Barataria Bay, Louisiana following the DWH spill with lung abnormalities as compared to dolphins sampled in Sarasota Bay, Florida. The Barataria Bay dolphins were 5-fold more likely to have moderate to severe lung disease, consisting of increased occurrence of alveolar interstitial syndrome, lung masses, and pulmonary consolidation. Corresponding to data from live health assessments, bottlenose dolphins stranding in the years immediately following the spill displayed a higher prevalence of primary bacterial pneumonia



compared to non-exposed dolphins (22 vs. 2%) (Venn-Watson et al. 2015). A study among the same study animals using a noninvasive technique for detection of exhaled breath metabolites found a significant relationship between pulmonary consolidation and metabolites consistent with chronic inflammation as evidenced by products of lung epithelial cellular breakdown and arachidonic acid cascade metabolites (Pasamontes et al. 2017). Pasamontes et al. (2017) concluded that the increased degree of cellular breakdown products in the Barataria Bay dolphins were consistent with, but not specific to, the proposed mechanism of chronic lung disease from aspirating or inhaling noxious agents.

### **Humans**

**Respiratory system abnormalities.** Several studies investigated the effect and risk of inhaled oil spill emissions on the respiratory system in humans following the DWH event. The DWH Oil Spill Coast Guard Cohort Study (DWH-CG), a survey-based study of US Coast Guard personnel involved in the response (Rusiecki et al. 2018), determined that more than half of study participants had been exposed to the oil (Alexander et al. 2018). Coughing was the most prevalent symptom reported (19.4%), followed by shortness of breath (5.5%) and wheezing (3.6%). An exposure–response relationship was evident between deployment duration and all three symptoms before the well was capped but only for coughing and wheezing post-capping. A similar pattern was noted for self-reported exposure to crude oil via inhalation as well as from other routes of exposure. Rates of reported respiratory symptoms were markedly stronger when participants reported exposure to both oil and dispersant compared to oil alone. This large study (n=4,855) was able to control for the effects of personal protective equipment (PPE) and other exposures not often included in such retrospective surveys. In prospective, longitudinal analyses utilizing health encounter data to calculate incidence of respiratory conditions in the 5 years following the oil spill, Rusiecki et al. (2020) noted elevated incidence of sinusitis, bronchitis, asthma, and respiratory

symptoms such as dyspnea, shortness of breath, and cough among responders exposed via inhalation to oil compared to those not exposed.

Gam et al. (2018a, 2018b) investigated effects of inhalation oil exposure by measuring lung function using spirometry in 7,775 adults enrolled in the GuLF STUDY, who participated in the response or who received training but were not hired. Overall, data demonstrated no marked difference between the two groups. However, workers that handled oiled plants/wildlife or recovered dead animals exhibited lower spirometry performance than unexposed workers. Gam et al. (2018c) also found that workers with high potential exposure to burning oil displayed reduced lung function 1 to 3 years after the oil spill based upon health encounter data, but these effects were not apparent 4–6 years later (Gam et al. 2018b; Lawrence et al. 2020). These findings were similar to those from the US Coast Guard cohort (Rusiecki et al. 2018) in which chronic respiratory conditions, overall and specifically asthma, were significantly elevated in responders reporting crude oil exposure compared to those with no exposure in the 2 year period post-spill.

Of the community-based human studies, Peres et al. (2016) evaluated 2,126 adult women living in Southern Louisiana and noted significant associations between high exposure levels to DWH oil and all 13 physical health symptoms assessed, with the strongest associations found for burning in the nose, throat, or lungs, sore throat, dizziness, and wheezing (all odds ratios > 4). The women most financially impacted by the oil spill were significantly more likely to report wheezing and other respiratory issues such as a stuffy, itchy, or runny nose. The Gulf Coast Health Alliance: Health Risks related to the Macondo Spill (GC-HARMS) longitudinal study of the communities impacted by the spill used a self-reporting approach to understand the physical and mental health issues (Croisant et al. 2017). Impacts on respiratory health were not reported separately but were included as part of the multiple descriptive indicators of general health. Lung function, as assessed by physicians, was not significantly different among the variably exposed communities studied. A GC-HARMS follow-up

study employed sophisticated analytical methods to examine seafood samples collected over three years to address gaps in knowledge associated with human risk assessment due to petrogenic PAH toxicity and communicated the findings to the stakeholder communities involved in the longitudinal study (Jackson et al. 2019).

### **Cardiovascular system**

Effects induced by pollutants that target the heart might be functional, such as lasting only for the duration of exposure, or irreversible, where the risk of which increases with dose and duration of exposure. Consequently, changes in the heart might result in both acute and chronic effects on an individual's fitness.

#### **Fish**

**Cardiac failure in early life stages.** Developing fish are particularly vulnerable to the effects of dissolved PAHs at the low ppb range, and thus early life stages may be widely impacted following major oil spills (e.g., Travers et al. 2015). Following the DWH spill, research focused on determining the mechanisms of previously observed cardiotoxicity on early life stages (Incardona 2017). Brette et al. (2014) assessed the impact of field collected DWH oil samples on *in vitro* cardiomyocyte preparations dissociated from the hearts of adult blue fin tuna (*Thunnus orientalis*) and yellowfin tuna (*T. albacares*). Four environmental samples were used and included source oil, artificially weathered oil (by heating at 90° to 105°C) and two skimmed oil samples. The approach was to examine the effects of these solutions on excitation-contraction (EC) coupling using electrophysiological and Ca<sup>2+</sup> imaging techniques. Effects on action potential duration in cardiomyocytes were observed, which correlated closely with the concentrations of three-ring PAHs in the samples. The functional effects on rhythmicity suggested that components of crude oil interfere with EC coupling, which links electrical excitation to contraction in cardiomyocytes. DWH oil exerted significant effects on K<sup>+</sup> channel inhibition and on the voltage-dependent properties of cardiac Ca<sup>2+</sup> channels. These effects on the ion channels that are important in the contractility of cardiomyocytes may indicate the mechanism

underlying toxicity in fish embryos. Following these suggested modes of action, studies showed that DWH oil impaired contraction of myocytes isolated from mahi-mahi at low PAH concentrations (Heuer et al. 2019), which explains observations of reduced stroke volume and diminished cardiac output in mahi-mahi and cobia (*Rachycentron canadum*) (Cox et al. 2017; Nelson et al. 2017).

MicroRNA (miRNAs) play a key role in embryogenesis and in regulation of other processes. Exposure to weathered and non-weathered oil suggested that slick oil more differentially altered miRNAs expression in mahi-mahi than treatment with source oil, regardless of developmental stage (embryonic vs larval development stages) (Diamante et al. 2017). In the analysis of the targets, disruption of the cardiovascular system pathways at later developmental stages was highlighted. Comparable miRNA changes were also observed in a similar exposure study of larval red drum where target genes were also identified as being those involved in development and functioning of the nervous system (Xu et al. 2019). This approach, using next generation sequencing and in-depth bioinformatics analyses, found that targets were predominantly involved in neuro-cardio system development processes and associated key signaling pathways such as axonal guidance signaling, cAMP-response-element-binding protein signaling in neurons, calcium signaling, and nuclear-factor-of-activated T cells signaling in cardiac hypertrophy. Significant cardiac morphologic effects were seen consistent with differentially expressed gene transcripts, particularly late in development (Xu et al. 2017). In addition, alterations in cardiac gene expression in developing mahi-mahi exposed to slick oil, but not source oil, were consistent with phenotypic changes such as reduced heart rate and pericardial edema (Xu et al. 2016).

Following the findings that crude oil disrupts EC coupling in developing fish hearts, Sorhus et al. (2016) examined the influence of dispersed oil on cardiac function and morphogenesis, characterized novel craniofacial defects, and investigated associated gene expression. Even short duration exposures at low concentrations were sufficient to

produce cardiac and craniofacial abnormalities. The specific nature of the abnormalities suggested that the target may be precursor cells in which ion channels are directly or indirectly affected. The data appear to support a unifying hypothesis involving depletion of intracellular calcium that results in several downstream organogenesis pathways being disrupted.

Cardiovascular stress may be assessed using morphometric analyses of heart form and function (Incardona, Collier, and Scholz 2004). Edmunds et al. (2015) found that mahi-mahi embryos exposed to field-collected DWH oil during embryogenesis exhibited contractility, looping, and circulatory defects. The type and degree of morphometric and cardiac-specific molecular responses was dose-dependent with differences in sensitivity due to age.

**Cardiac function.** In addition to cardiac failure, effects of oil on cardiac function may also be temperature dependent, as Perrichon et al. (2018) found that higher temperatures resulted in greater number of bradycardias in larval mahi-mahi but reduced the occurrence of hematoma and severity of edema.

Effects on fast-swimming predatory fish were of particular concern due to the high aerobic demand of species such as tuna and amberjack (Incardona et al. 2014). These species also displayed concentration-dependent effects on cardiac function and heart failure with circulatory disruption, pericardial edema, and other secondary malformations. These effects were independent of the consequences of weathering on the DWH oil to which the embryos were exposed. In a study with mahi-mahi, Esbaugh et al. (2016) reported that the weathering increased oil toxicity and that pericardial edema correlated with acute lethality.

Cardiac effects were detectable even at low exposure levels (<1 ppb) in some species, suggesting low thresholds for developmental cardiotoxicity in fish (Incardona et al. 2015). Even transient embryonic exposure at low concentrations was sufficient to induce delayed toxicity. Adult zebrafish exposed to oil as embryos also showed subtle changes in heart shape and swimming performance (Hicken et al. 2011). Common toxicological responses in the cardiovascular system were seen in radically different species of fish larvae. For example, cardiac

output was reduced by 70% in larval red drum (Khursigara et al. 2017), driven by reduced stroke volume rather than bradycardia. The results also demonstrated that sensitivities and target organs were similar in this estuarine species as were found in pelagic fish.

**Consequences of cardiac damage.** The physiological outcomes of changes in cardiac development and cardiovascular function were investigated in mahi-mahi. Swimming performance was affected in both juvenile and adult fish (Mager et al. 2014, 2018; Stieglitz et al. 2016a) likely through reductions in myocyte shortening, stroke volume, cardiac output, and stroke work (Nelson et al. 2016). Data demonstrate that oil exposure can result in sublethal cardiac effects in adult pelagic fish.

Kirby et al. (2019) found that acute crude oil exposure altered mitochondrial function and adenosine diphosphate (ADP) affinity in cardiac muscle fibers from mahi-mahi. These observations are in contrast to studies on red drum cardiac muscle (Johansen and Esbaugh 2019) where no marked effect on mitochondria were detected, although effects on red muscle mitochondria were reported (see metabolism section below).

## **Birds**

**Cardiac structure and function.** Oral and dermal dosing studies in double-crested cormorants, laughing gulls, western sandpipers, and homing pigeons (*Columba livia*) were conducted to assess the physiologic effects of artificially weathered DWH oil (Bursian et al. 2017a). Following observation of enlarged, flaccid hearts during necropsy of the cormorants, laughing gulls, and homing pigeons, cardiac biomarkers were included in the suite of endpoints for dermal exposure of cormorants to artificially weathered DWH oil. Oil exposure induced significant systolic myocardial dysfunction as assessed with echocardiography (Harr et al. 2017c). It was concluded that the changes were indicative of a possible dilated cardiomyopathy. Arrhythmias, particularly bradycardia, were found in all treated birds. Cardiac damage to fish affecting the EC coupling mechanism and calcium cycling, combined with the significant rise in plasma-ionized calcium in

the oil-exposed birds may suggest a common mechanism that warrants further investigation as a pathogenic mechanism of disease. Not only did DWH oil initiate cardiac abnormalities in susceptible developing life stages, as demonstrated in fish, but the oil also produced clinically significant alterations in adult birds after three weeks exposure (Harr et al. 2017c). Among double-crested cormorants orally dosed with 5 or 10 ml artificially weathered DWH oil kg/body weight/day for 21 days or exposed by 6 dermal applications of 13 ml oil to breast and back feathers every 3 days, gross cardiac lesions including thin walls and flaccid musculature were detected in both groups of birds and myocardial fibrosis in a few of those dermally exposed (4 out of 13). At no time was myocardial infarction found upon complete necropsy of all birds in the studies.

### **Marine Mammals**

Although there were no published studies of the cardiovascular system in marine mammals exposed to DWH oil at the time of the workshop, a few studies have been completed in the last year. Linnehan et al. (2020) found that marine mammals (managed in San Diego) and wild common bottlenose dolphins (in Sarasota, Florida and Barataria Bay, Louisiana) displayed a similarly high prevalence (approximately 90%) of systolic heart murmurs, with maximal intensity typically in the sternal cranial and left cranial areas of the heart. However, three dolphins in Barataria Bay also exhibited medium mitral regurgitation and moderate to severe lung disease; veterinarians gave all three individuals a guarded to poor prognosis based upon all of the health assessment data available. In a separate study using echocardiography, oil-impacted dolphins in Barataria Bay showed several differences in cardiac structure, including thinner interventricular septa, thinner left ventricular walls, smaller left atria, and higher prevalence of tricuspid valve prolapse and thickening (Personal communication, B. Linnehan). Veterinarians diagnosed two of the Barataria Bay dolphins with pulmonary arterial hypertension. Dead, stranded dolphins from areas affected by DWH oiling also exhibited a higher rate ( $p = 0.002$ ) of cardiac fibrosis (46%) compared to stranded dolphins at locations outside of the DWH oil spill

footprint (19%) (Personal communication, B. Linnehan). It is unclear whether cardiac morphometric abnormalities/lesions are driven directly by oil exposure, or as secondary effects from inhaled/aspirated oil damaging the lungs (or some combination of both).

### **Humans**

**Cardiac function.** In humans, some preliminary findings are suggestive of an elevated risk of cardiac effects, with increases in palpitations and chest pain. In a self-reporting health study (Croisant et al. 2017), the proportion of respondents reporting hypertension was higher in the oil-exposed communities than the control community. Strelitz et al. (2018) noted that individuals working on the spill for more than 180 days and who stopped working due to heat exhibited an increased risk of non-fatal heart attack. After 5 years, DWH oil spill workers exposed to total hydrocarbon levels  $>0.3$  ppm displayed a 62–81% higher hazard for heart attack compared to workers with the lowest exposure levels (Strelitz et al. 2019b). Further, residential proximity to the spill and duration of clean-up work were associated with a suggested 29–43% higher hazard of heart disease events (Strelitz et al. 2019a).

#### **Cardiovascular conditions and symptoms**

The DWH-CG Study evaluated longer term cardiovascular conditions among Coast Guard responders. In prospective, longitudinal analyses using health encounter data to calculate incidence of respiratory conditions in the 5 years following the oil spill, Denic-Roberts et al. (2020) demonstrated elevated incidence of cardiovascular conditions, such as hypertension and palpitations, among responders exposed via inhalation to oil, compared to those not exposed.

### **Central nervous system (neurological and sensory including behavioral and psychological)**

The influence of oil on the central nervous system (CNS) can be profound since the consequences impact so many downstream effectors and sensory functions. These include neurobehavioral impacts,



effects on the senses of smell and sight, potential of neuronal injury, and psychological impacts (at least in humans).

### **Fish**

**Behavior.** Using male Siamese fighting fish (*Betta splendens*), Bautista et al. (2019) demonstrated that a 4-week dietary exposure with DWH oil produced an increase in aggressive behavior, as well as decrease in nest building, testis mass, and brain mass compared to unexposed fish. When exposed to WAFs, fish including red drum, Atlantic croaker, and coral reef fish from the *Pomacentridae* and *Lethrinidae* families showed elevated risk-taking behavior, an impaired ability to capture prey, reduced sheltering behavior, diminished social behavior, and decreased voluntary movement speed and distance traveled (Armstrong et al. 2019; Johansen and Esbaugh 2017; Rowsey et al. 2019). Transcriptomics studies identified potential mRNA and microRNA pathways that are consistent with neurological and sensory disruptions, including disrupted structure and function of synapses and nervous tissue (Xu et al. 2019, 2017).

**Olfaction.** There were clear effects of oil exposure on olfaction in fish (Cave and Kajiura 2018), an area largely unexplored prior to the DWH oil spill. Studies in Atlantic stingrays (*Hypanus sabinus*) reported an average decrease in magnitude of the olfactory response by 45.8% after 48 hr exposure to oil mimicking the concentrations found in the coastal regions. Olfaction in this species is particularly important in all aspects of their life history, including feeding and mate and predator detection. Behavior and olfaction following oil exposure were also examined in bicolor damselfish (*Stegastes partitus*); chemical alarm cue was used to test the response of exposed and unexposed fish (Schlenker et al. 2019b). Controls avoided the cue as expected, whereas exposed fish did not. In addition, the response to several cues was assessed using an electro-olfactogram. Exposed fish were less likely to detect the chemical alarm cue; however, the amplitude or duration was not markedly affected when they did

respond. The implications of this investigation are that predator avoidance behavior might be modified by oil exposure in the wild. In a similar study with mahi-mahi, unexposed juveniles avoided high concentrations of DWH oil (27.1 µg/L), but exposed individuals did not. As with damselfish, oil exposure did not significantly affect amplitude or duration of response as measured by the electro-olfactogram (Schlenker et al. 2019a).

**Vision.** mRNA and miRNA expression analysis in several marine species exposed to slick oil reported impacts on pathways involved in visual function. Disruption of these pathways affected retinal histology as well as visual acuity (as determined by optomotor response) in a PAH dose-dependent manner in mahi-mahi (Xu et al. 2018), red drum, and sheepshead minnows (Magnuson et al. 2018). A subsequent study using slick oil and zebrafish determined that disruption of these gene expression pathways and visual acuity were consistent with a dose-dependent loss of glial cells supporting the neuronal network in the retina (Magnuson et al. 2020). These data suggest that exposure to oil may disrupt eye development and function across a number of fish species.

### **Birds**

**Behavior.** Perez et al. (2017a, 2017b, 2017c) using homing pigeons as a surrogate for migratory birds, demonstrated that flight behavior might be altered following oil application to feathers. Homing pigeons were trained to a variety of flight scenarios. Flight behaviors were examined before and after oil application of artificially weathered DWH oil on flight and tail feathers (20% total coverage and equal to only 1% of the bird's body weight). For the longest flights studied (161 km) (Perez et al. 2017b), after a single oil application, pigeons showed a 2.6-fold rise in flight duration compared to flights prior to oil application, and their flights took longer than control birds during the experimental flights. The distance flown by oiled birds was on average 28 km greater than controls and showed decreased route efficiency. Perez et al. (2017b) suggested that this altered behavior may be due to the potential for increased lift along a ridgeline and to reduce exposure to predation. In a

second study using a shorter distance and additional post-oiling flights, Perez et al. (2017c) noted changes in flight behavior that continued for 35 days post-oiling, including more time spent stopped (approximately 3-fold greater compared to pre-oiling). In pigeons oiled multiple times, Perez et al. (2017a) found longer flight times compared to baseline pre-oiling flights and reduced weight between flights due to lower food consumption. Data suggest reduced refueling capacity (see section on metabolism below), but may also be attributed to more time spent preening during their downtime, as is common for lightly oiled birds. The results indicate that light oiling might affect behavior in order to compensate for altered integrity of flight feathers.

Maggini et al. (2017b) studied the effects of oiling on takeoff of western sandpipers following light oil application to wing and tail feathers. This application represented 20% of total body surface but only 5% of visible oil surface of the bird with wings folded and was used to mimic a potential oiling event initiated by a bird landing on water. Maggini et al. (2017b) employed accelerometry to determine that, in the first 0.4 sec of flight, the distance traveled by oiled birds was 29% less than prior to oiling and at 10° less of an angle. Maggini et al. (2017b) postulated that this slowdown might make these individuals more prone to predation than unoiled conspecifics and result in the use of less productive, but safer, refueling sites, further diminishing the likelihood of successful migration.

### **Turtles**

**Behavior.** It is not just the chemical toxicity of oil that results in impact to oil-exposed organisms: physical miring in oil might also severely hinder movement, leading to behavioral changes and alterations in predator/prey relationships and ultimately mortality (reviewed in Wallace et al. 2020). During the DWH incident, sea turtles were found physically coated by oil with heavy fouling – the most readily apparent and immediate harmful effect observed (*Deepwater Horizon* Natural Resource Damage Assessment Trustees 2016; McDonald et al. 2017b; Stacy 2012). Indeed, survival of turtles fouled at the highest category documented was determined to be unlikely without intervention

(*Deepwater Horizon* Natural Resource Damage Assessment Trustees 2016; Mitchelmore, Bishop, and Collier 2017).

### **Humans**

**Psychological effects.** The psychological effects of the DWH oil spill on humans was the subject of several key studies involving both community exposed and occupationally exposed cohorts. Bell, Langhinrichsen-Rohling, and Varner (2018) reported that post-traumatic stress symptoms (PTSS) increased rapidly in the Gulf coast community and was higher among those who noted direct contact with the oil compared to those with no contact (Cherry et al. 2017). A longer-term study, involving 314 participants interviewed directly following the spill and at the second-year time point, indicated that the occurrence of mental health symptoms including depression and PTSS did not diminish over time and were similar at both time points. Mental health issues and modest decreases in neurobehavioral functions were also detected in the Gulf Long-term Follow-up Study (GuLF STUDY) (Kwok et al. 2017; Quist et al. 2019) that involved 8,969 clean-up workers and 2,225 non-workers, and adverse mental (and physical) effects were significantly worse for participants that were exposed to both the DWH oil spill and Hurricane Katrina (Lowe et al. 2019).

The DWH-CG Study evaluated acute neurological and mental health symptoms among Coast Guard responders during their response to the DWH oil spill. Increased prevalence of headaches, lightheadedness, difficulty concentrating, numbness/tingling sensation, blurred vision, and memory loss/confusion was associated with elevated frequency of crude oil exposure via inhalation and via skin contact (Krishnamurthy et al. 2019; Rusiecki et al. 2018). Exposure to both crude oil and oil dispersants yielded associations appreciably greater in magnitude than for crude oil alone (Krishnamurthy et al. 2019). Wang et al. (2020) found elevated prevalence of anxiety and depression among responders reporting longer oil spill response work duration and intensity, increasing exposure to crude oil, and physical symptoms.

The Women and Their Children's Health Study (WaTCH) administered telephone interviews to a population-based sample of 2,842 women who were questioned regarding their mental health and domestic conflict as well as their exposure to the oil spill (Rung et al. 2016). Over 28% reported symptoms of depression, 13% severe mental distress, and 16% a rise in the number of fights with their partners with an associated increase in the intensity of those conflicts. Reported exposure to the DWH oil spill was a significant predictor of these outcomes. Mental health issues and memory loss were also significant issues among wives of clean-up workers (Rung et al. 2015). In a latent profile analysis of post-traumatic stress disorder (PTSD) in women from the WaTCH study, Nugent et al. (2019) found that women in the more symptomatic classes were more likely to have been impacted by the DWH oil spill compared to asymptomatic classes.

### **Gastrointestinal system**

The gastrointestinal tract (GIT) is the target organ for many ingested pollutants, with a large surface area where mucosal and deeper damage might occur. Substances absorbed through the GIT then travel through the bloodstream and possess the potential to exert adverse effects on other systems.

### **Birds**

Common loons (*Gavia immer*; caught in coastal Louisiana from 2011 to 2015) with higher blood PAH concentrations had lower body mass compared to birds with lower circulating PAH concentrations (Paruk et al. 2016). Evidence indicated that this may be attributed to nutrient malabsorption but suggested that more data are needed due to species differences in the tolerance and metabolism of PAH compounds. While not assessed directly, Dean et al. (2017) suggested that oral exposure of cormorants to artificially weathered DWH oil might impact the GIT resulting in malabsorption based upon reduced feed intake and body weights. In addition, Harr et al. (2017b) reported anecdotal evidence of abnormal excreta in the orally- and dermally-dosed cormorants, indicating that oil impacts absorption and integrity of the GIT.

### **Turtles**

Oil ingestion in sea turtles was documented during the DWH incident rescue efforts (Stacy 2012). Previously, retention times of oil in the GIT of turtles was estimated to be approximately 9 days but this study demonstrated that it was <48 hr in Kemp's ridley and loggerhead sea turtles (Mitchelmore et al. 2015). The estimated amounts of oil in the upper digestive tract of these turtles were used to calculate environmentally relevant daily doses (100 or 1,000 mg/kg) for a surrogate sea turtle oil toxicity study (Mitchelmore 2012a; Mitchelmore, C. L. 2012b). In this study juvenile snapping turtles and red-eared sliders were orally dosed daily (100 or 1,000 mg/kg) to the oil commonly fingerprinted coating sea turtles from the DWH incident, namely, Slick A oil, for 14 days. Diagnoses were based upon individual organism evaluation of hematological, blood chemistries, and histological data and showed evidence in the snapping turtles of treatment related dehydration, GI protein loss, or malabsorption, and in the red-eared sliders there were some cases of moderate subacute gastritis/gastroenteritis in the 1,000 mg/kg slick A DWH treatment group (Mitchelmore et al. 2015). In addition, Harms et al. (2019) reported significant differences between treatments and controls (with greater differences in the combined oil/dispersant exposures) in a number of relevant clinical chemistries analytes in experimentally-exposed hatchling loggerhead sea turtles, including total protein, albumin, globulin, potassium, and sodium, reflecting loss of electrolyte and hydration homeostasis consistent with decreased seawater consumption and dehydration. Harms et al. (2019) also noted a failure to gain weight in dispersant- and oil/dispersant combination-exposed hatchlings. In contrast to the DWH laboratory oil studies, many of the listed effects were not observed in loggerhead and Kemp's ridley sea turtles exposed to crude oil during the DWH spill and/or may be discerned from capture and transport stress (Stacy 2012; Stacy et al. 2017).

### **Humans**

The DWH-CG study reported a significant elevation (prevalence ratios > 1) in several acute gastrointestinal symptoms following exposure to oil including nausea, diarrhea, stomach pains, and constipation (Rusiecki et al. 2018). Interestingly, the prevalence ratios were higher,



particularly for diarrhea, stomach pains, and constipation, among those exposed to both oil and dispersant.

### **Hepatic system**

As the site of detoxification, the liver is often damaged by exposure to pollutants, including oil. Liver injury covers a wide range of effects including hepatic necrosis, lipid accumulation (steatosis), or hepatobiliary dysfunction.

### **Fish**

Menhaden (*Brevoortia* sp.) exposed to DWH oil in Barataria Bay (n=18) exhibited increased prevalence of hepatic lesions involving necrosis and hypertrophy compared to those inhabiting a control region (n=16) in Delaware Bay, New Jersey (Bentivegna et al. 2015). In 48% of the Barataria Bay fish, there was evidence of fibrosis not detected in control fish. Bentivegna et al. (2015) concluded that liver and stomach lesions are indicative of exposure over a longer period and the potential for permanent damage, reducing their overall survival probability. Jones et al. (2017) also demonstrated that hepatic gene expression patterns were significantly changed in sheepshead minnows experimentally exposed to different WAFs.

A multi-year study of coastal fish [red snapper (*Lutjanus campechanus*) and gray triggerfish (*Balistes capriscus*)] between 2011 and 2014 found elevated hepatic biomarkers including ethoxyresorufin O-deethylase (EROD), glutathione transferase, and glutathione peroxidase activities (Smeltz et al. 2017). Levels of EROD activity declined over the 4-year period with livers from gray triggerfish exhibiting signs of fatty liver and fluorescent substances similar to PAHs.

Juvenile southern flounder experimentally exposed to a sediment-oil mixture for 32 days displayed histopathologic changes in liver (and gill) tissues of fish exposed to more than 8 mg/kg tPAH<sub>50</sub> (the sum of 50 individual PAHs) with signs of hepatic intravascular congestion and vacuolation (Brown-Peterson et al. 2015b).

### **Birds**

Changes in liver oxidative stress endpoints (i.e., total antioxidant capacity) were reported in western sandpipers dosed orally with artificially weathered DWH oil (1 or 5 ml/kg bw/day for 20 days). Absolute liver weights in oil-exposed sandpipers were also higher compared to controls (Bursian et al. 2017b). Data suggested that the sandpipers increased their overall antioxidant capacity in the liver in response to oil exposure. Increased liver weights in response to oil exposure was detected in cormorants and laughing gulls, suggesting elevated weight is the result of compensatory metabolic responses (Harr et al. 2017b; Miller, Peakall, and Kinter 1978; Peakall et al. 1989).

Horak et al. (2017) observed hepatotoxic effects of ingested oil on laughing gulls that were orally dosed with artificially weathered DWH oil (5 or 10 ml/kg bw/day) for 27 days. Hepatic total glutathione, oxidized glutathione, and reduced glutathione rose significantly, as did relative (% body weight) liver weights. Double-crested cormorants orally dosed with artificially weathered DWH oil (5 or 10 ml/kg bw/day) over a 21-day period showed dose-related elevation in total glutathione, oxidized glutathione, and reduced glutathione, while superoxide dismutase and glutathione peroxidase were significantly reduced. Further, these birds exhibited significant decreases in other liver enzyme activities and clinical chemistry endpoints indicative of diminished hepatic function (Dean et al. 2017). Livers collected at necropsy from these birds showed induction of both CYP1A4/1A5 enzymes as measured by assays for benzyloxyresorufin O-debenzylase (BROD), EROD, methoxyresorufin O-demethylase (MROD), and pentoxyresorufin O-depentylase (PROD) (Alexander et al. 2017).

Double-crested cormorants exposed to oil via feather preening demonstrated abnormal plasma markers associated with liver function compared to orally dosed birds, as well as markedly enlarged livers (Cunningham et al. 2017; Dean and Bursian 2017; Harr et al. 2017b). Dermal exposed birds were estimated to have consumed approximately 0.9 ml of artificially weathered DWH oil/kg bw/day, which was significantly lower than the dose levels for the oral dosing study, and thus differences in hepatotoxicity may be attributed to dose effects.

### **Turtles**

Hepatic exposure and effects of DWH oil were evident in some field-collected sea turtles, as bile metabolite levels were elevated (Ylitalo et al. 2017; Ylitalo, Collier, and Stacy 2015). Similar dose-dependent elevations in bile metabolites were also seen in both species in the surrogate study (Mitchelmore et al. 2015). Further, a rise in bile protein concentrations in snapping turtles exposed to 1,000 mg/kg slick A DWH oil suggested that there may have been reduced intake or assimilation of food (Mitchelmore et al. 2015). A 14 day-exposure to slick A oil at 1,000 mg/kg resulted in significant elevations in oxidative stress and antioxidant compensatory responses in the liver tissue in red-eared sliders evidenced as increases in total antioxidants and total, reduced, and oxidized glutathione levels. Only elevations in total antioxidant levels were evident in liver tissue of the snapping turtle, highlighting difference between turtle species (Mitchelmore et al. 2015). Interestingly, the lack of compensatory antioxidant responses may be the reason why there was evidence for oxidative damage in this species.

### **Marine Mammals**

There was some evidence of effects of oil exposure on the hepatobiliary system in bottlenose dolphins (Schwacke et al. 2014). Abnormal values for two or more of the liver enzymes measured were reported among 19% of the dolphins live captured in Barataria Bay, LA compared to none detected among those captured in Sarasota Bay, FL. Toxic liver damage was also suspected in several bottlenose dolphins that stranded in the oil spill footprint following the spill (Venn-Watson et al. 2015). However, the prevalence of dolphins with hepatobiliary effects decreased over time and were not significantly different in the exposed compared to unexposed dolphins captured in 2013 and subsequently (Smith et al. 2017).

### **Integumentary system**

The skin is vulnerable to the toxic effects of chemicals through direct contact. It is the barrier against many external pollutants and whilst it works effectively against some, other substances may penetrate quite readily. However, following the DWH spill few studies focused on dermal effects.

### **Fish**

There is some evidence that DWH oil contamination correlates with increases in skin lesions in some fish species. Anecdotal reports from fishermen motivated Gulf-wide cruises by Murawski et al. (2014), which documented an elevated prevalence of skin lesions in red snapper and other GoM fish species near the DWH wellhead compared to later years. During laboratory exposures of juvenile southern flounder, Bayha et al. (2017) noted bloody lesions on and near fins only in fish exposed to oil-laden sediment, but sample size was small and did not quantify the prevalence.

### **Birds**

Bird feathers are uniquely adapted for flight, buoyancy, and body temperature maintenance. Constant preening helps to maintain integrity of flight, contour, and down feathers. Oil not only damages feather integrity, but can also act as a skin irritant (Jessup and Leighton 1996) and produce behavioral changes in preening. Infra-red imaging and implanted body temperature transmitters were used to determine how repeated application of DWH oil to the feathers of double-crested cormorants affected body temperature regulation (Cunningham et al. 2017). Matted feathers and loss of feather integrity were observed following the first oil application. Skin discoloration was found by the 2<sup>nd</sup> to 3<sup>rd</sup> application, and extensive feather plucking, particularly of down feathers, was noted in the majority of oil-exposed cormorants after the final oil application (Cunningham et al. 2017). The skin at necropsy was discolored and thickened, but it was not possible to assess adequately by standard histopathologic techniques. Forward-looking infrared (FLIR) images taken of the front and back of oiled and control birds throughout the study showed that thermal conductance (heat loss) increased on the head, neck, back, and breast of oiled birds over the course of the study, indicating both the spread of oil over the body with preening and detrimental effects of loss of down feathers and feather integrity of contour feathers on heat loss in this diving bird.

Horak et al. (2020) externally oiled ring-billed gulls (*Larus delawarensis*) with weathered DWH oil, with 7 ml applied to the breast, wing tips, and tail feathers for three consecutive days, and

collected feathers. Thermography imagery was collected weekly for 4 weeks to investigate feather structure (quantified using a barbule clumping index) and thermoregulatory ability (characterized by internal body temperature and external surface temperature). Post-oiling feather clumping was significantly higher in oiled and rehabilitated (that is washed with detergent on day 8 or 9) birds compared to controls, but steadily declined over time in both groups. However, feather microstructure in rehabilitated birds was indistinguishable from controls within three weeks of washing, whereas the feathers of oiled birds were still significantly clumped a month post-oiling. External temperatures for rehabilitated birds did not differ from controls within a week of rehabilitation.

### **Marine Mammals**

Recently, Wise et al. (2018) used WAF of Alaskan oil and CEWAF to determine that although WAF was not cytotoxic to sperm whale (*Physeter macrocephalus*) skin cells *in vitro*, there was some chromosomal damage. CEWAF was more cytotoxic and genotoxic than WAF, which appeared to be due to a rise in total levels of PAHs in CEWAF than WAF. Studies conducted prior to the DWH oil spill demonstrate that oil acts as a skin irritant in cetaceans (Jessup and Leighton 1996).

### **Humans**

Effects of DWH oil on the skin were investigated among workers in direct contact with oil during the clean-up operations. Prevalence of acute dermal symptoms including skin rashes or itching was elevated among responders with increasing exposures to crude oil (PR=1.87; 95% CI: 1.45-2.40 for high vs low crude oil exposure). The risk of long-term dermal conditions in the 2 years post-DWH was elevated in oil spill responders vs. non-responders (RR=1.21) (Rusiecki et al. 2018).

### **Immune system**

The immune system can be highly sensitive to exposure from pollutants resulting in increased susceptibility to infection, induced allergies, and autoimmunity. A conceptual model of the immune

system, its components, and where impacts of oil exposure were reported is depicted in Figure S4 (Supplementary Information).

### **Fish**

**Host resistance.** The effects of oil on the immune system were studied in red snapper, both alone and in conjunction with exposure to the fish pathogen *Vibrio anguillarum* (Rodgers et al. 2018b). The expression of five immune-related gene transcripts were investigated in various combinations with/without oil and with/without pathogen. All five immune genes were upregulated in the oil-exposed groups after 8 days exposure but were downregulated or displayed no marked differences with controls after 10 or 17 days. These immune-associated gene expression levels fell sharply from day 8 to day 10, with only one cytokine gene remaining up-regulated until day 17. Rodgers et al. (2018b) emphasized the need to understand the many factors that modulate immune gene expression and whilst some responses may be conserved among species, there are likely to be species differences. Nevertheless, if oil affects the immune response to bacterial infection, particularly across multiple immune pathways, the outcomes for individual animals and populations are likely to be critical.

Bayha et al. (2017) conducted a similar study with southern flounder but exposing juvenile fish to oiled sediment with/without *V. anguillarum*. Flounder exposed to oil prior to bacterial challenge exhibited a 94% mortality within 48 hr whereas those challenged with the pathogen only had a < 10% mortality. These results were linked to oil-induced immunosuppression: the oil-exposed cohort demonstrated reduced levels of the major fish antibody class, IgM, and an overall downregulation of genes involved in immune function, response to stimulus, and hemostasis. Thus, PAHs sequestered in sediments may exert long-term adverse health impacts on benthic species.

**Genetic markers of immune effects.** Jones et al. (2017) exposed sheepshead minnows to two concentrations of HEWAF, a chemically enhanced fraction, and Corexit 9500 dispersant for 7 and

14 days. Immune-related gene sub-networks were impacted across all treatment groups, especially the high HEWAF group. In the 7-day treatment, most pathways were down regulated but in the 14 day experiment the opposite was the case. Innate immune processes were most affected, such as macrophage and granulocyte function. To a lesser extent some pathways relating to T and B cell functioning were also affected.

### **Birds**

Oil-induced oxidative damage of proliferative hemoprogenitors in bone marrow has long been documented in mammals and, more recently, decreased cell mediated immune response was noted in birds (Olsgard et al. 2008). Dean et al. (2017) reported that double crested cormorants dosed orally with artificially weathered DWH oil demonstrated a significant decrease in total white blood cell, heterophil, and eosinophil concentrations during the course of the 21-day study. However, other white blood cell types were not markedly different between exposed and unexposed birds and reduced in both groups. In the same study, the oral high-dose group consistently showed smaller and fewer inflammatory lesions throughout the body, including kidneys, liver, heart, pancreas, and throughout the GIT than did either the control or low-dose group (Harr et al. 2017b). Briggs, Gershwin, and Anderson (1997) suggested that oil toxicity exerts more impact on cell-mediated immune responses than antibody-mediated responses; however, in this study only measurements of cell-mediated immune function were white blood cell and differential counts. Thus interpretation of the specific effects of oil are somewhat limited. The design of the study limited assessment of other immune system endpoints. For example, changes in lymphocyte concentrations in the avian exposure investigations were noted but not explored thoroughly with proliferation and function studies. Further research is warranted.

### **Turtles**

Clinicopathologic abnormalities were studied in experimentally exposed hatchling loggerhead sea turtles that included some immune cell

parameters (Harms et al. 2019). No significant differences were found in total white blood cell or differential white cell counts among treatment groups, although this study was necessarily limited to small sample sizes that may have restricted the power to detect effects.

### **Marine Mammals**

**Immune function.** Immune responses, both through functional *in vitro* assays and blood analyses from exposed dolphins, were notably associated with exposure to DWH oil in live captured bottlenose dolphins (De Guise et al. 2017). There was a consistent increase in T and B lymphocyte proliferation among animals in Barataria Bay, LA compared to those in Sarasota Bay, FL. Although cytokine concentrations were not significantly different, some key patterns were compatible with responses found in other oil-exposed species. The balance of cytokines suggested a Th2 response consistent with intra-cellular infections and changes in B cell functions that may affect responses to extra-cellular bacterial infections. The largest effects were seen among dolphins from Barataria Bay that was sampled in 2011 with evidence of a reduction in those effects with time since the spill.

*In vitro* exposure studies were also carried out on lymphocytes from bottlenose dolphins to elucidate the effects of oil and dispersant on different lymphocyte subtypes as markers of innate and adaptive immunity (White et al. 2017). Both T and B cell proliferation were enhanced following exposure to oil at the higher levels. In the chemically enhanced mixtures, the opposite response was detected. The combination of responses led White et al. (2017) to conclude that adaptive immune reduction may result in enhanced susceptibility to infection but that elevated natural killer cell activity may be in some way advantageous in increasing viral surveillance ability.

### **Circulatory system**

Hematotoxicity may be initiated by many different pollutants resulting in reduction in the production and function of the oxygen-carrying red cells: the erythrocytes.



## **Fish**

**Effects on erythrocytes.** Fifty-seven adult, mixed sex red drum, caught in six oil-contaminated and two reference sites where no oil was observed along the Louisiana coastline were sampled for blood analysis 2 years after the oil spill (Harr et al. 2018). A reference interval for red drum packed cell volume was established (42–62%) from fish at the two reference sites, and 18% of the fish sampled at contaminated sites exhibited anemia (<42%), while no anemic fish were captured at the reference sites, 2 years after the oil spill when little to no oil was visible.

## **Birds**

**Effects on erythrocytes.** Anemia and abnormal erythroid morphology were reported in double-crested cormorants experimentally exposed to artificially weathered DWH oil both orally and dermally (Harr et al. 2017a). Anemia was mildly regenerative but inadequately compensated, indicating decreased bone marrow production. This study documented that avian Heinz body structure and/or organelle damage is significantly structurally different than found in mammals. The classic button appearance of mammalian Heinz bodies is almost never found in birds even in blood samples from birds with biomarkers indicating severe oxidative damage (Bischoff, Harr, and Barron 2021). Therefore, confirmation by electronic microscopy is often required. Collecting anticoagulated whole blood samples in 2–3% glutaraldehyde is recommended because such samples, if appropriately stored in a cool dark place, can be archived for years. Further, coagulopathy, suspected due to lack of clotting noted at necropsy in orally dosed laughing gulls and cormorants both orally and dermally exposed to oil was confirmed using activated clotting times in double crested cormorants and pigeons dosed with artificially weathered DWH crude oil. Coagulopathy results in blood loss through the GIT (hematochezia) and other vascular leakage, which might directly result in anemia. Often with extravascular hemorrhage, protein values decrease when compensated. Fallon et al. (2018), using a less rigorous measurement technique reported that American oystercatchers (*Haematopus palliatus*), black skimmers, brown pelicans, and great egrets (*Ardea alba*) captured in

the months following the DWH oil spill exhibited signs of oxidative injury to erythrocytes as indicated by the presence of Heinz bodies. Packed cell volume (of circulating erythrocytes) was lowered by as much as 19% compared to reference populations and there was evidence of a regenerative hematologic response as indicated by increases in reticulocytes by as much as 40% compared to reference populations. Further, birds with no visible oiling captured in the impacted area displayed apparent anemia suggesting that it was not possible to assess oil-induced effects from visible signs of oiling alone.

## **Turtles**

**Effects on erythrocytes.** Previous studies of petroleum toxicity in field or laboratory experiments demonstrated alterations of some blood cell parameters (Lutcavage et al. 1995; Vargo et al. 1986). However, no apparent evidence of hemolytic anemia was observed in oiled DWH turtles (Stacy 2012; Stacy et al. 2017). Further, there was no evidence of anemia or abnormal erythroid morphology in any of the turtles in the DWH oil surrogate turtle study (Mitchelmore et al. 2015). All turtles (control and oiled groups) showed evidence of inflammation that may have been due to the gavage tube used to conduct the oral dosing and thus all inflammatory responses were removed from consideration due to this complication.

## **Marine mammals**

**Effects on erythrocytes.** Schwacke et al. (2014) noted some cases of anemia and inflammation in dolphins from areas affected by DWH oiling. Decreased hemoglobin levels in 4 out of 32 dolphins in oiled Barataria Bay, Louisiana were found compared to 0 out of 26 dolphins in unoiled Sarasota Bay, Florida. The reticulocyte counts of the anemic dolphins were all greater than the maximum count from the Sarasota Bay population.

## **Metabolism, energetics and biomechanics**

An outcome of the systemic toxic effects of oil exposure, particularly damage to the cardiovascular system, may be downstream impacts on metabolic rate and energetics with indirect consequences for survivorship.

## Fish

**Oxygen consumption and metabolic demand.** Oil and ultraviolet (UV) exposed mahi-mahi embryos displayed enhanced rates of oxygen consumption for extended periods prior to hatching compared to controls (Pasparakis et al. 2017). There was also a significant correlation between oxygen consumption, embryo sinking rates, and onset of negative buoyancy.

Johansen and Esbaugh (2019) exposed red drum to PAH mixtures for 24 hr and compared results to samples from unexposed control fish. It was postulated that the reductions in critical swim speed despite maintenance of maximum metabolic rate in exposed fish were due to impaired mitochondrial function in swimming and cardiac muscles. Whilst Johansen and Esbaugh (2019) found no evidence for effects of mitochondrial dysfunction in cardiac muscle, impairment was observed in red muscle. Oil exposed mitochondria from red drum muscle tissue suggested a reduction in ATP generation. However, doses used to induce impairment were at the upper end of the range reported during the DWH oil spill. Chub mackerel (*Scomber japonicus*) exposed to weathered oil as a WAF for 72 or 96 hr were studied in a swim tunnel respirometry system (Klinger et al. 2015). Energetic demand increased in all fish in response to oil exposure at 96 hr. Evidence indicated that the rise in metabolic demand might be attributable to energetic cost of detoxification. However, studies with mahi-mahi (Mager et al. 2014; Stieglitz et al. 2016b), cobia (Nelson et al. 2017), and red drum (Johansen and Esbaugh 2017) showed no marked impact of oil exposure on resting or standard metabolic rate.

Social competition was affected by crude oil exposure in red drum through reduced aerobic scope (the difference between basal metabolism and maximum capacity for oxygen transport, i.e., standard metabolic rate compared to maximum metabolic rate) (Khursigara, Johansen, and Esbaugh 2018). This endpoint is predictive of social dominance in these gregarious fish and fish exposed to oil were more likely to be subordinate than expected demonstrating that physiological constraints affect social status and behavior with secondary effects on ecological fitness

## Birds

**Energetic effects.** The energetic costs of migratory flights may be extremely high particularly for small migratory birds such as the western sandpiper that breed in the Arctic and overwinter in coastal habitats of North, Central and South America (Morrison et al. 1993; Nebel et al. 2002). In sandpipers with artificially weathered DWH oil applied to their feathers, Maggini et al. (2017a; 2017c) found that light and moderate oil exposure increased metabolic cost of flight up to 22% and 45% respectively compared to control birds. Flight control decreased which would result in elevated duration of migration and might alter reproductive failure and mortality rates. In terms of kinematic parameters, light and moderately oiled birds flying slowly in a wind tunnel had larger wingbeat amplitudes than controls, while moderately oiled birds showed greater wingbeat frequencies at all flying speeds due to poorer lift and increased drag. Maggini et al. (2017b) estimated that this enhanced energetic cost may result in stopover delays of approximately 1 to 8 days per stopover for a total potential delay of 9 to 45 days for western sandpipers to reach their Arctic breeding grounds. If oil ingestion caused impairment of GIT function (Leighton 1993), this delay would be further exacerbated and might exert catastrophic population impacts due to reproductive failure at the breeding grounds.

Multiple metabolic pathways associated with energy production were impaired in double-crested cormorants dermally exposed to oil such that 20% of their surface area was lightly oiled (Dorr et al. 2019). Both plasma and liver metabolomes were affected compared to controls indicating potential impacts to thermoregulation, cardiac function, and hematologic parameters. Modeling of seasonal changes in the thermoregulatory cost of oiling in double-crested cormorants based upon oil application to feathers (Cunningham et al. 2017) demonstrated that the resting metabolic rate (RMR) following application of 13 to 78 g of artificially weather DWH MC252 rose by 31 to 76% over winter and 29 to 73% during the breeding season (Dorr et al. 2020). Although body weight of cormorants oiled in the lab might be maintained by increased food consumption (Cunningham et al.

2017), wild birds were estimated to require more than 2 hr per day of additional foraging time (Dorr et al. 2020).

Basal metabolic rates (BMRs) of western sandpipers at 27°C and metabolic rates under thermal stress conditions (sliding scale down to -3°C) over a 12 hr period were tested in respirometry chambers. Oil applied to the back and breast feathers to a total of 20% body coverage did not result in significant changes in thermal conductance, but birds that were oiled 3 days before testing exhibited lower body temperatures at the time of testing and lost more body weight than other birds. In addition, birds exposed to oil for 3 days before respirometry displayed changes in blood chemistries consistent with damage to the loop of Henle, evidenced as increased urea, decreased sodium, and albumin, as well as increases in markers of oxidative stress including reduced and total glutathione, superoxide dismutase, and malondialdehyde  $\pm$  4-hydroxyalkenals.

Using homing pigeons as a surrogate for the effects of DWH oil on bird flight, Perez et al. (2017b) reported that birds externally oiled with artificially weathered DWH oil either once or multiple times, (1) took significantly longer to return home, (2) lost more weight during flight, (3) were unable to replenish their energy stores despite adequate food supply, and (4) showed reduced body weight over time. Body weight was likely lower due to both enhanced expenditure of energy in longer flights with damaged feather integrity and decreased calorie intake due to diminished appetite and possible compromised GIT function. The conclusion was that even partially oiled birds were likely to display poorer body condition and an elevated risk of reproductive failure and subsequent mortality.

Ecosystem damage at staging sites is a vital issue for migratory species such as sanderlings (*Calidris alba*) and red knots (*C. canutus*), as refueling affects individual fitness, reproductive success, and survival during migration. These species were the subject of a study into differences in fuel loads and fueling rates at two sites, one of which contained PAH-contaminated sediments (Louisiana). Plasma metabolite profiles and fattening index scores indicated that pre-migratory fueling rates were slower

for sanderlings in Louisiana, but not for red knots; however, both species departed the PAH contaminant sites later than normal. Multiple factors such as migration patterns, food supply, and other contaminants may also have played a role. These conclusions were supported by the results of a dosing study in which sanderlings were administered no, low, medium, or high doses of PAH ranging from 0 to 1260  $\mu$ g total PAH/kg bw/day (Bianchini and Morrissey 2018). Consistent with other studies, mass gain was found to be lower compared to controls in all groups.

Bonisoli-Alquati et al. (2020) used a microarray transcriptomic approach in liver samples to investigate genome-wide gene expression changes in wild Seaside Sparrows (*Ammodramus maritimus*) exposed to oil in saltmarshes. Data demonstrated that as well as inducing liver hypertrophy, energy homeostasis was impacted through changes in gene expression encoding molecules associated with energy regulation and lipid biosynthesis.

## Summary and Conclusions

When organisms are exposed to chemical contaminants, the resulting adverse effects can manifest in a variety of ways depending on the species, life-stage, individual life history, nature of the exposure, and countless other variables. Despite this variation, there is consistency among the DWH toxicity testing and field observation results including data from DWH NRDA field studies, the DWH literature (laboratory and field studies) outside of the NRDA program, and the established oil/petroleum toxicity literature beyond DWH.

- From a taxonomic perspective, many of the mechanisms of action and the resulting disease pathways are conserved across species and taxa. For example, the effects on cardiovascular development, structure, and function, and the reports of cardiac symptoms and disease appear similar across multiple taxa
- From a toxicologic perspective, there is a logical progression following exposure routes leading to molecular and cellular damage/mechanisms that manifest as organ disease,



finally resulting in reduced fitness, growth, reproductive potential, and survival. For example, flounder exposed to oil in the sediment have reduced antibody production, are more susceptible to infection, demonstrate reduced growth and oxygen transport capacity, can develop skin lesions, and eventually die due to the combined effects of DWH oil exposure, immune dysfunction, and a bacterial challenge.

- From a clinical perspective, the breadth of adverse health effects and clinical signs associated with DWH oil exposure are due to various combinations of molecular/cellular damage in multiple organ systems. By applying the knowledge gained from sublethal studies of the pathogenesis of oil-related disease, further impacts from oil exposure can be better understood. For example, HPA axis abnormalities and adrenal gland damage in bottlenose dolphins result in abnormal steroid hormone concentrations and electrolyte imbalances, and likely contribute to immunosuppression and reproductive failure.

It is clear that not every organism exposed to oil will experience all of the adverse health effects presented in the results summarized above. It is likely that each individual might suffer from various combinations and severities of negative impacts, some more detrimental than others. However, all the organisms exposed to DWH oil may be forced to allocate resources to deal with the spill-related injuries. Some will eventually recover, but some may succumb to their injuries, potentially impacting their population. Laboratory results and field analyses demonstrate that exposure to DWH oil might result in a suite of mechanistically derived toxicological responses and impairments to homeostasis occurring across multiple taxa, resulting from the conserved physiological processes across vertebrate taxa. Although not the focus of the workshop or this review, workshop participants did note that many physiological systems are conserved between invertebrate and vertebrate taxa, and that there is a good deal of research on invertebrates that is consistent with the research discussed here.

Cardiovascular effects were one of the most apparent common endpoints across taxa – most intensely studied in fish, but also reported for birds and emerging evidence for effects in mammals. Of note are recent studies showing similar findings between marine mammals and humans for cardiovascular effects. In addition, oxidative stress and damage endpoints were also a commonly observed effect. The finding of impaired stress response in marine mammals prompted investigations across other taxa, resulting in positive findings for an association in birds and fish.

The large body of work that has been published on the toxic effects of oil on vertebrates since the DWH oil spill has made a major contribution to the toxicological knowledge base. The field studies were often based in the same regions, the Gulf States most impacted by the oil, particularly Louisiana and Mississippi allowing for greater comparability. Investigators examined effects at all trophic levels, often within the same food chain. The associated experimental exposure, and many of the *in vitro* studies, were also closely linked to the fieldwork using oil collected during the spill, both early in the event and following weathering. This also increased confidence in the comparability of the results.

The findings (summarized in [Table 1](#)) indicate some critical commonalities and mechanisms that are also likely to be linked across systems, particularly through impacts on signaling processes. These data may be useful in identifying and informing data gaps regarding oil toxicity in other, less-studied taxa/species. The pathways summarizing how the reproductive, endocrine, respiratory, and immune systems are impacted are illustrated in Supporting Information Figures S1 – S4. Evidence from the 5 taxa provides new information on reproductive, endocrine, respiratory, and immune effects. Evidence of impacts on the other systems remain important and should not be excluded when considering the likely effect of future oil spills. This summary puts the pathways for effects into the vertebrate-wide context where the evidence-base is most compelling.

This synthesis indicates that petroleum and its many constituents, through direct as well as indirect routes of exposure, when released into the marine environment produce widespread adverse

health effects at both the individual and population levels. Notwithstanding the caveats associated with making comparisons across taxa where the nature of exposure including oil type, concentration/dose, duration, and route could be the reasons for observed differences whereby effects are seen in one taxon but not in another, the pathways and toxicological impacts of DWH oil can result in long-term changes in the abundance and distribution of marine wildlife.

The workshop participants also discussed key outstanding data gaps in our collective understanding of the toxicology of oil in vertebrates, as we attempt to move on from DWH and prepare for the next oil-related disaster. The questions included:

- How do we simultaneously address the concepts of environmental exposure (e.g., detecting dose and its patterns) and evaluating toxic exposure for injury/damages to natural resources (e.g., correlating exposure with biological endpoints/biomarkers) during an event?
- How do we ensure that we have appropriate baseline (i.e., pre-spill) data? This was identified as an especially difficult challenge for assessing human exposure and toxic effects, given the difficulty in ramping up research studies in the wake of DWH (although the DWH-CG Study has been able to account for preexisting conditions via health encounter data).
- How can managers comprehensively characterize the type, magnitude, and duration of ephemeral exposures during future oil spills/events as quickly as possible, rather than opportunistically “chasing the oil”?
- How can researchers and resource managers compare/replicate laboratory exposures with inferences and limited data sets on field conditions/exposures during an event?
- How do researchers balance studying/interpreting acute observations with inferences about chronic effects from prior/ongoing exposures? How can they determine what is truly an acute observation when signs/symptoms and disease are compounded by chronic effects?
- How do we move from characterizing and quantifying sublethal impacts to population-level effects? How does the field integrate work from toxicologists and population modelers? Can cross-taxa studies help inform how individuals’ exposure to oil may impact behavioral and social structures?
- How does mild, moderate, and severe multi-organ systems damage impact overall health, survival, and reproduction?
- How do we best approach standardized, comparable field studies with multiple stressor exposure scenarios with both natural and anthropogenic stressors?
- How should managers and researchers update the pre-DWH conceptual model of dispersants with the post-DWH research on both dispersant toxicity and dispersed oil toxicity?
- How can negative results from laboratory and field studies be appropriately reported and integrated into our collective understanding of oil toxicity?
- Can we develop standards for surveys and health assessments of wildlife during and after an oil spill, especially in the context of characterizing and quantifying NRDA injuries and losses?

The interpretation of toxicological effects following such large-scale events requires scientific inference. Our ability to assess the injuries across an ecosystem in the wake of environmental disasters is naturally limited in terms of timing, spatial coverage, our existing knowledge of the contaminants and affected resources, our lab and statistical capabilities, budget, and political will. Therefore, in order to describe and estimate the potential total impacts of toxic releases, scientists and policy makers must combine the available site-specific data with the literature to draw taxonomic and mechanistic/functional inferences about effects that may not be directly studied (or where studies resulted in inconclusive findings). As many of these systems are conserved across taxa, our ability to postulate based upon conserved physiologic systems across taxa while considering the unique biological aspects of organisms is critical to characterizing a holistic assessment of impacted

resources. Future studies to investigate the commonalities and differences in oil toxicity among vertebrates require robust combinations of study designs involving laboratory-, field-, and survey-based approaches.

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## Declaration of Interest and Disclaimers

No potential competing interest was reported by the authors. All opinions expressed in this paper are the authors' and do not necessarily reflect the policies and official views of the Uniformed Services University, the Department of Defense, the United States Coast Guard, the Department of Homeland Security, or the National Oceanic and Atmospheric Administration.

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