Chapter 29
Digging Deeper than LC/EC50: Nontraditional Endpoints and Non-model Species in Oil Spill Toxicology

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Abstract The response to the 2010 *Deepwater Horizon* (DWH) oil spill lead to a number of peer-reviewed publications examining the effects of the released oil and dispersant on fish species found in the northern Gulf of Mexico (GoM). Many of these papers, for very good reasons, focused on assessing toxicity by defining lethality through identification of dose-response curves that were specific to a given species, age class, exposure type, oil preparation method, and many other factors. Often those dose-response curves were used to predict LC or EC50 concentrations – amounts of oil that produced an effect on 50% of the exposed organisms. The advantage of this approach is obvious, in that it provides a single point estimate and variance of a concentration required to produce a given effect. This point estimate can then be compared across different exposure regimes to compare susceptibilities. Relevant LC/EC50 data is summarized and discussed in *A synthesis of DWH oil, chemical dispersant and chemically dispersed oil aquatic standard laboratory acute and chronic toxicity studies* (see Mitchelmore et al. A synthesis of Deepwater Horizon oil, chemical dispersant and chemically dispersed oil aquatic standard laboratory acute and chronic toxicity studies (Chap. 28). In: Murawski SA, Ainsworth C, Gilbert S, Hollander D, Paris CB, Schlüter M, Wetzel D (Eds.) Deep oil spills – facts, fate and effects. Springer; 2020). By constraining toxicity to this point estimate (often of lethality), however, researchers run the risk of missing effects that evince more subtle effects that do not manifest themselves as overt mortality in the short term. In the present chapter, we focus exclusively on fish and explore some of

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these endpoints, many of which were successfully used in recent years to assess sublethal health impacts on marine fish as part of the response to the DWH spill. We compare what is known about differences in sensitivity, among species, and between age classes within species, examining both organismal and molecular endpoints. Developmental impacts on cardiac health, swim performance, and sensory systems have been widely studied. We discuss what is known about effects on fish immune and endocrine function, the microbiome of the intestine and gill, and intracellular effects such as altered gene expression, oxidative stress, and DNA damage. In conclusion, we attempt to compare the endpoints, assess the sensitivity and utility, and link molecular- and individual-level impacts to larger population and community-level effects.

Keywords ELS (early life stage) · Cardiotoxicity · CEWAF (chemically enhanced water accommodated fraction) · WAF (water accommodated fraction) · Toxicity testing · Microbiome · Immune function · RNA sequencing

29.1 Introduction

The response to the 2010 *Deepwater Horizon* (DWH) oil spill lead to a number of peer-reviewed publications examining the effects of the released oil and dispersant on fish species found in the northern Gulf of Mexico (GoM). Many of these papers, for very good reasons, focused on assessing toxicity by defining lethality through identification of dose-response curves that were specific to a given species, age class, exposure type, oil preparation method, and many other factors. Often those dose-response curves were used to predict LC or EC50 concentrations – amounts of oil that produced an effect on 50% of the exposed organisms. The advantage of this approach is obvious, in that it provides a single point estimate and variance of a concentration required to produce a given effect. This point estimate can then be compared across different exposure regimes to compare susceptibilities. Relevant LC/EC50 data is summarized and discussed in *A synthesis of DWH oil, chemical dispersant and chemically dispersed oil aquatic standard laboratory acute and chronic toxicity studies* (see Mitchelmore et al. 2020). By constraining toxicity to this point estimate (often of lethality), however, researchers run the risk of missing effects that evince more subtle effects that do not manifest themselves as overt mortality in the short term. In the present chapter, we focus exclusively on fish and explore some of these endpoints, many of which were successfully used in recent years to assess sublethal health impacts on marine fish as part of the response to the DWH spill. We compare what is known about differences in sensitivity, among species, and between age classes within species, examining both organismal and molecular endpoints. Developmental impacts on cardiac health, swim performance, and sensory systems have been widely studied. We discuss what is known about effects on fish immune and endocrine function, the microbiome of the intestine and gill, and intracellular effects such as altered gene expression, oxidative stress, and

DNA damage. In conclusion, we attempt to compare the endpoints, assess the sensitivity and utility, and link molecular- and individual-level impacts to larger population and community-level effects.

29.2 Test Organism Sensitivity

29.2.1 Species-Specific Differences in Sensitivity to Oil Exposure

Parallel studies, using identical WAF preparations and exposure conditions, of acute toxicity to larval fish have revealed substantial differences among species (McKim et al. 1985) underscoring the range of sensitivities in different species and the importance of selecting the optimal test organism. In these studies of acute toxicity, 7-day-old inland silverside (*Menidia beryllina*) was more sensitive than 3-day-old sheepshead minnow (*Cyprinodon variegatus*) and more sensitive than <1-day-old Gulf killifish (*Fundulus grandis*) (Finch et al. 2017). These apparent differences in sensitivity should be viewed in the context of the age of the test organisms. Age, and presumably size, confers a higher tolerance to acute exposures to oil as illustrated on studies of bay anchovy exposed to HEWAF preparations of Macondo oil. These 24-h exposure studies on bay anchovy established LC50 values of 9.71 μg/L TPAH for embryos, compared to 690 and 1610 μg/L TPAH for 5- and 21-day-old larvae, respectively (Duffy et al. 2016; O'Shaughnessy et al. 2018). Thus, the observation that older silverside are more sensitive than the newly hatched killifish further underscores the significant difference in sensitivity among organisms. Although other factors may be at play, sensitivity differences among species may be a reflection of differences in PAH metabolism. In a DWH oil intraperitoneal injection (IP) study assessing species-specific PAH metabolism differences of subadult red drum (*Sciaenops ocellatus*), Florida pompano (*Trachinotus carolinus*), and southern flounder (*Paralichthys lethostigma*), analysis of PAH bile metabolites indicated that pompano exhibited a much faster rate of PAH metabolism than red drum and southern flounder. Results suggest that pompano may be better able to depurate toxic PAH compounds (Pulster et al. 2017).

29.2.2 Differences in Sensitivity Among Life Stages

Early life stages are generally more sensitive to environmental insults than older conspecifics which form the foundation for acceptance of early life stage toxicity testing as a faster and more cost-effective alternative to full life-cycle tests. In most cases, early life stage testing accurately predicts outcomes of full life-cycle tests (McKim 1985). Size is undoubtedly an important factor determining differences in

sensitivity within and among species, likely because size dictates metabolic demands and thereby needs for exchange of gasses and metabolic products as well as surface area/volume ratios (Bianchini et al. 2002; Grosell et al. 2002). The general relationship of smaller early life stage organisms being more sensitive than older and larger conspecifics holds true also for oil/PAH exposures as illustrated in the following. However, it is important to note that impacts to several endpoints, such as sensory function, aerobic scope, and reproduction, cannot be assessed in early life stages and that these endpoints in some cases appear highly sensitive (Stieglitz et al. 2016; Xu et al. 2018; Johansen and Esbaugh 2017) and very well may be of relevance for population-level impacts. Further, several studies have revealed that early life stage exposures may lead to effects that are not readily evident until later in life (Xu et al. 2018; Mager et al. 2014; Magnuson et al. 2018) underscoring the importance for studies also on later life stages.

29.3 Targets of Oil Toxicity

29.3.1 Cardiac Developmental and Functional Effects on ELS

Cardiac development and function in embryo and larval fish from the GoM have been demonstrated to be sensitive to crude oil exposure. The early life stages (ELS) of yellowfin tuna (*Thunnus albacares*) were found to display pericardial edema and reduced heart rate following 48 hrs of exposure to HEWAFs at EC50 concentrations of 2.5 and 7.5 μg/L∑50PAH, respectively (Incardona et al. 2014). Similarly sensitive are mahi-mahi (*Coryphaena hippurus)* embryos and larvae with 48-hr EC50s ranging from >5.1 to 7.3 μg/L∑50PAH for HEWAFs and 11.3 to 13.0 μg/L∑50PAH for CEWAFs of slick, source, and weathered oil (Esbaugh et al. 2016). As with yellowfin tuna, mahi-mahi displayed pericardial edema but also presented an altered atrium-ventricle angle and evidence of reduced cardiac contractility (Edmunds et al. 2015), the later suggesting reduced stroke volume. The coastal red drum is no less sensitive with a pericardial edema 48-h EC50 of 2.4 μg/L∑50PAH for HEWAF (Khursigara et al. 2017). For the first time in any oil-exposed fish ELS, red drum stroke volume was measured to reveal that this parameter is far more sensitive than heart rate with the two factors both contributing to cardiac output with a 48-h EC50 of 2.2 μg/L∑50PAH for HEWAF (Khursigara et al. 2017). While the aryl hydrocarbon receptor (AhR) plays a role in the pericardial edema phenotype (Incardona et al. 2006, 2011) observed in ELS fish following oil exposure, AhR-independent effects have also been reported (Incardona et al. 2005). In mahi-mahi, as well as red drum, pathway analyses of RNA-seq experiments revealed cardiac Ca^{2+} homeostasis as a target for crude oil toxicity (Xu et al. 2016, 2017a, b). Impaired Ca^{2+} homeostasis in myocytes may certainly account for observations of reduced cardiac contractility and stroke volume (Edmunds et al. 2015; Khursigara et al. 2017) and has also been implicated in cardiac hypertrophy (Xu et al. 2017b).

29.3.2 Noncardiac Developmental Effects on ELS

In addition to cardiac impairments, several other biological systems have demonstrated sensitivity to oil exposure. Mahi-mahi embryos exhibit yolk sac edema when exposed to a low concentration (1.2 μg/L ∑50PAH) of HEWAF for 48 hrs (Pasparakis et al. 2016), and decrease in yolk sac depletion (nutrient uptake) was observed in embryos exposed to higher concentrations (14.9–26.6 μg/L ∑50PAH) of HEWAF for 24 hrs (Mager et al. 2014). Other embryonic developmental impairments observed in sheepshead minnows when exposed to 257 μg/L ∑50PAH of CEWAF for 48 hrs were retarded development and decreases in eye pigmentation and movement (Bosker et al. 2017). Both length and weight of larvae of several fish species appeared to be influenced by exposure to CEWAF or HEWAF. Bosker et al. (2017) observed a reduction in standard length sheepshead minnow larvae exposed for 48 hrs to 257 μg/L ∑50PAH CEWAF. A reduction in lenght was also seen in spotted seatrout exposed for 96 hrs (at much lower concentrations) of approximately 74 μg/L ∑50PAH exposure solutions, whether HEWAF or CEWAF (Brewton et al. 2013), whereas a marked reduction in weight was measured in bay anchovy exposed for 24 hrs to 0.24–1.23 μg/L ∑50PAH of CEWAF (Duffy et al. 2016). Data from RNA-seq pathway analysis of oil-exposed larval mahi-mahi indicated that several other biological systems were affected by a 48-h exposure to DWH source oil (4.6 μg/L ∑50PAH) or DWH slick oil (12 μg/L ∑50PAH) HEWAF (Xu et al. 2016). Particularly affected were genes involved in neurodegeneration of the central nervous system, eye degeneration, visual impairment, and abnormality of the vertebral column. These studies highlight some of the potential consequences of oil exposure on multiple biological systems leading to the likely increase in post-exposure mortality.

29.3.3 Cardiac Function and Swim Performance in Later Life Stages

While cardiotoxicity has long been a recognized effect of oil exposure in developing fish, it was not until recently that the developed heart of adult fish was also identified as a target for oil exposure. Yellowfin and bluefin tuna (*Thunnus thynnus*) myocytes exposed to crude oil solutions showed prolonged action potentials through blockage of the delayed rectifier potassium current and also decreased Ca^{2+} current and Ca^{2+} cycling leading to excitation-contraction uncoupling (Brette et al. 2014). Although it is unknown at present how blood plasma PAH concentrations relate to environmental concentrations, the effects of PAH exposure on isolated tuna myocytes were observed at low μg/L ∑50PAH concentrations in the bathing salines, suggesting that impacts to cardiac function may occur during environmentally realistic oil exposures. The first evidence of oil-induced impacts to cardiac function following exposures of adult GoM species came from a study exposing mahi-mahi to HEWAF

dilutions of <10 μg/L ∑50PAH (Stieglitz et al. 2016). This study reported reduced maximal oxygen uptake, reduced aerobic scope, and a concomitant reduction in critical swimming speed (U_{crit}) with no apparent effect on gill histology after only 24 hrs of exposure in young adult mahi-mahi. Subsequent studies employing vascular blood flow probes inserted into anesthetized mahi-mahi revealed normal heart rate but greatly reduced stroke volume and thereby reduced cardiac output when exposed for 24 hrs to HEWAF at ~ 10 μg/L ∑50PAH prior to experimentation (Nelson et al. 2016). Similar exposures resulted in reduced U_{crit} for cobia (*Rachycentron canadum)* and a substantial reduction in stroke volume, although the later was largely compensated for by an elevated heart rate (Nelson et al. 2017). Further studies on cobia employing 24-h HEWAF exposures at ~ 22 μg/L ∑50PAH found reduced cardiac power output (Cox et al. 2017), although this effect could be mitigated by β-adrenergic stimulation alluding to possible compensations in intact animals. In addition to the cardiac impacts noted above for pelagic GoM species, the coastal red drum show reduced aerobic scope and U_{crit} following 24 hrs of acute exposure to HEWAF at 12 μg/L ∑50PAH. These impairments remained for 8 weeks after the 24-hr exposure attesting to the persistence of cardiac effects induced by oil exposure to adult GoM species (Johanson and Esbaugh 2017).

Interestingly, reduced swim performance has been observed in oil-exposed GoM species without a concomitant reduction on maximal oxygen uptake or aerobic scope. For red drum, a 24-h exposure to HEWAF at 4 μg/L ∑50PAH resulted in both reduced burst swimming speed (U_{burst}) and U_{crit} without any apparent reductions in aerobic scope (Johanson and Esbaugh 2017). Similarly, 48-hr HEWAF exposures to mahi-mahi embryos at 1.2 μg/L ∑50PAH followed by rearing in uncontaminated water for 30 days resulted in reduced U_{crit}, again without reductions in aerobic scope or maximal oxygen uptake (Mager et al. 2014). The observed effects on swim performance in absence of notable reductions on oxygen supply points to non-respiratory pathways for PAH impacts on swim performance. Three such possible nonexclusive pathways include (1) impaired neuromast function leading to reduced swimming efficiency (Johanson and Esbaugh 2017), (2) reduced efficiency of mitochondrial ATP production, and finally (3) impacted calcium cycling in skeletal muscle (Johanson and Esbaugh 2017) as seen for cardiomyocytes (Brette et al. 2014, Xu et al. 2016, 2017a, b).

29.3.4 Impacts on Sensory Systems and Behavior Including Prey-Predator Interactions

A recent study (Rowsey et al. 2019) on red drum determined that larvae exposed to HEWAF at ~25 μg/L ∑50PAH for 24 hrs, 21-day post fertilization (dpf), displayed increased thigmotaxis (time spent in open areas as opposed to in shelter) leading to an increased exploration of foraging area. Despite exploring larger foraging areas, oil-exposed red drum larvae (24 hrs at 35 dpf) caught less prey in a time-limited

prey capture assay at ~55 μg/L∑50PAH (Rowsey et al. 2019). Similar behavioral impacts have been observed for a number of larval great barrier reef fish species exposed to HEWAF for 24 hrs (Johansen et al. 2017). In mesocosm experiments, larval reef fish showed altered habitat preference, increased exploration of different habitats (more movement), and less tendency for shoaling (lower average group size) following 24 hrs of exposure to 5.7 μg/L∑50PAH (Johansen et al. 2017). When exposed to predators under simulated natural conditions, these oil-induced behavioral changes in larval fish resulted in increased predation mortality (Johansen et al. 2017). While integrative observations of behavioral changes may reflect impaired sensory function, impaired central nervous system function, or both, RNA-seq studies on mahi-mahi as well as red drum have revealed that brief exposures during larval development may alter expression of genes related to sensory function. For mahi-mahi, 24–96 hrs of exposure to HEWAFs at concentrations as low as 7 μg/L∑50PAH showed developmental impacts on the peripheral nervous system through exposure to oil. In particular, eye development and the development of eye function were among the pathways most impacted by oil exposure (Xu et al. 2016). Similar findings were reported for red drum larvae exposed to HEWAF at ~5 μg/L∑50PAH for 24–72 hrs where pathway analyses pointed to likely impacts to the nervous system (Xu et al. 2017b). These observations of gene expression changes were paralleled by observations of reductions in eye and brain areas (Xu et al. 2017b). Functional implications of these gene expression and developmental impacts have been noted using an optomotor response to assess eye function. As a consequence of exposure during embryonic development, larval sheepshead minnow show reduced eye function following exposure to HEWAF at 159 μg/L∑50PAH (and higher) (Magnuson et al. 2018). The same assay indicated that red rum larvae exposed during embryonic development were more sensitive, displaying reduced eye function following HEWAF exposure at 2.7 μg/L∑50PAH (Magnuson et al. 2018). However, larval mahi-mahi exposed to HEWAF during embryonic development were even more sensitive, showing effects at the low levels of 0.67 μg/L∑50PAH (Xu et al. 2018). With the exception of one study on great barrier reef species (Johansen et al. 2017), it is unclear to what extent impaired sensory function and altered central nervous system function may affect overall performance and survival under natural conditions. However, considering the effect thresholds for impaired eye function and the apparent ubiquity of this response across species, these impacts may well influence population level responses to oil exposure.

29.3.5 Impacts on Microbiomes

In-depth analysis of the microbiome (defined loosely as the suite of microorganisms that are associated with a host organism in different organs) offers an intriguing opportunity to examine subtle effects of exposure that may, nonetheless, have important effects on the host organism. Several studies clearly demonstrated that an incursion of oil from the DWH spill altered the microbial community structure

in water and sediment (Hazen et al. 2010; Kostka et al. 2011; Mason et al. 2014), but only a handful of papers have examined this effect on marine fish species. Arias et al. (2013) and Larsen et al. (2015) examined the effects of oil exposure on the skin microbiome of the Gulf killifish and found no evidence for a linkage between oil exposure and skin microbiome shifts using culture-based approaches and ribosomal intergenic spacer analysis (RISA), although neither paper reported concentrations of PAHs in water or sediment where the samples were collected. In contrast, both Brown-Peterson et al. (2015) and Bayha et al. (2017) showed that laboratory exposure to oil-contaminated sediment (54 and 57 mg/kg $\sum$50PAH, respectively) altered the microbiome of both gills and intestines in southern flounder in ways that were clearly associated with the oil exposure, including a significant increase in the prevalence of bacteria linked to hydrocarbon degradation in upper gill, lower gill, and intestinal communities. Both studies used predictive metagenomics to show that KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways involved in contaminant degradation increased in abundance following oil exposure and pathways involved in biosynthesis of metabolically important compounds were strongly decreased in abundance following exposure to oil-contaminated sediments.

The effect of oil exposure on the commensal microbiome is likely worthy of further study as there is a clearly demonstrated linkage between intestinal Ahr receptor ligands and the intestinal microbiome in vertebrates. In particular, Ahr activation has been demonstrated to play a strong role in mediating tetrachlorodibenzofuran contaminant-driven shifts in intestinal microbiome structure in mice (Zhang et al. 2018). The idea that expression of certain proteins affected by oil exposure (such as Ahr) could influence the structure of the host microbiome has interesting consequences for studying the interaction of host and contaminants.

29.3.6 Impacts on Immune Function

The immune system of fish, like that of mammals, is comprised of innate and adaptive immunity. Innate immunity is considered the first line of defense through physical barriers and nonspecific recognition of microbial molecular patterns, whereas adaptive immunity, the second line of defense, is comprised of humoral and cell-mediated immunity, in which specific antigen recognition leads to either B cell proliferation and antibody production or cytotoxic T cell proliferation. Both the innate and adaptive immune responses use a complex network of signaling molecules (cytokines, chemokines), specialized cells, and organs that work in concert to clear microbial pathogens and maintain immunological homeostasis (Rauta et al. 2012).

Oil exposure can have a direct effect on the immune system through biotransformation or detoxification systems such as the cytochrome P450 (CYP) enzyme system. These systems are shown to be interrelated and not exclusively independent. In

fish, immune organs such as the head, kidney, and spleen have the capacity to detoxify xenobiotics indicating a biotransformation system within the immune system (Reynaud et al. 2008). In addition, in the liver, PAH activation of the AhR and PAH metabolism by the CYP enzymes along with Ca^{2+} mobilization can induce immunotoxicity (Reynaud and Deschaux 2006).

Three field studies of adult Gulf killifish collected in 2010 from DWH oil-contaminated sites of the northern GoM indicated modulations to both innate and adaptive immunities had occurred. Modulation of the adaptive immune system included notable decreases of circulating lymphocytes and enlargement of splenic melano-macrophage centers (MMCs) in relation to increased activity of ethoxyresorufin-O-deethylase (EROD), an established biomarker for PAH exposure in the environment (Omar-Ali et al. 2014). In addition, RNA-seq analysis of liver tissue indicated upregulation of adaptive immune genes, IgM, and tumor necrosis factor receptors 14 and 21 (TNFr14 and 21), relative to upregulation of genes associated with hypoxia and the CYP/AhR pathway (Garcia et al. 2012). Whereas microarray analysis of gill tissue demonstrated modulation of innate immunity with enrichment of genes involved in wound healing, inflammation, and the acute phase response, there was also a correlation with enrichment of CYP genes that have oil-associated expression (Dubansky et al. 2013).

RNA-seq and microarray analyses revealed immunosuppression in the livers of GoM fish exposed to either oil or dispersed oil. In juvenile red drum exposed for 3 days to a CEWAF of 1.73 μg/L∑50PAH, differential gene expression analysis indicated the majority of immune genes were significantly downregulated. These included genes associated with granulocyte and macrophage function (23 genes), T cell receptor (4 genes), and B cell receptor (2 genes). This downregulation of immune genes further correlated to significant upregulation of CYP1A, GST, and UDP-glucuronosyltransferase genes involved in metabolism of xenobiotics (Wetzel DOI: 10.7266/NT8P5Z08). Altered immune processes were observed in adult sheepshead minnow exposed for 7 or 14 days to environmentally relevant HEWAF concentrations of 0.26–5.95 μg/L∑50PAH and CEWAF concentrations of 0.35–1.10 μg/L∑50PAH, with the most affected responses related to macrophage and granulocyte functions (Jones et al. 2017).

Following the DWH spill, offshore surveys of fish populations observed incidences of skin lesions in GoM fish especially in bottom-dwelling species near the DWH site (Murawski et al. 2014). The lesions were hypothesized to be caused by PAH suppression of the immune system allowing for opportunistic bacteria colonization. Bayhe et al. (2017) were able to demonstrate this phenomenon in juvenile southern flounder exposed to weathered DWH oil-contaminated sediments at a concentration of 57.40 mg/kg∑50PAH for 7 days, followed by a 1-h bacterial challenge with *V. anguillarum*, resulting in body lesions similar to those reported in the field. The observed lesions were most likely due to a reduction of IgM gene expression in the spleen and kidney of oil-exposed southern flounder. IgM is the main immunoglobulin in fish that is essential for clearance of bacterial pathogens.

29.3.7 Oxidative Stress and DNA Damage

Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the organism's ability to detoxify reactive intermediates, such as those generated by metabolism of PAHs through cytochrome P450. Depending on the severity of oxidative stress, this imbalance between excessive production of ROS and antioxidant defenses can lead to lipid peroxidation (LPO), resulting from a suite of chain reactions triggered by radicals causing loss of membrane integrity and DNA damage. Potential damage to DNA can be caused by a variety of mechanistic endpoints, such as oxidized bases, apurinic/apyrimidinic sites, single- or double-strand breaks, and formation of DNA adducts, in which PAH metabolites intercalate into the DNA (Xue and Warshawsky 2005; Starostenko et al. 2017).

Monitoring organismal antioxidant defenses as markers for oxidative stress due to PAH exposure in fish is relatively new. The antioxidant enzymes, superoxide dismutase (SOD), and catalase (CAT) activities have been extensively used as biomarkers for a variety of contaminants; however direct links could not be established for SOD-CAT activities to a specific class of contaminants including PAHs (van der Oost et al. 2003), rendering these ineffective as markers for PAH-associated oxidative stress. Reduced glutathione (GSH), an important non-enzymatic antioxidant, acts as a scavenger for ROS (particularly H_2O_2) and PAH reactive intermediates to produce oxidized glutathione (GSSG), which is then recycled back to GSH for antioxidant defense. The relationship of PAH exposure and associated oxidative stress has been established using GSH/GSSG for antioxidant defense assessments. Other useful assessments of oxidative stress include measuring levels of malondialdehyde (MDA; a by-product of LPO), lipid hydroperoxides, and the total antioxidant capacity (TAC). In nuclear and mitochondrial DNA, 8-hydroxy-2-deoxyguanosine (8-OHdG) is one of the predominant forms of free radical-induced oxidative lesions and it has been widely used as a biomarker for oxidative stress leading to DNA oxidative damage.

In a series of controlled DWH surrogate oil exposures, targeted GoM fish species exhibited an overall decrease in antioxidants, increase of lipid peroxidation, and evidence of DNA damage. A correlation of ∑50PAH in the liver and decrease in antioxidant defense was observed in Florida pompano exposed to CEWAF concentrations of 14.7 μg/L ∑50PAH (Wetzel DOI: 10.7266/N7PZ5793). Red drum experienced a significant reduction in antioxidant defenses, increase in lipid peroxidation, a decrease in total antioxidant capacity, and a slight (not significant) increase in liver DNA oxidative damage (8-OHdG) when exposed to 485.07 mg/kg ∑50PAH and 688.94 mg/kg ∑50PAH DWH surrogate oil-contaminated feed for 14 days (Wetzel DOI: 10.7266/N7Q23XRS). Similarly, effects of oxidative stress were observed in southern flounder exposed to 47.50 mg/kg ∑50PAH DWH surrogate oiled sediment for 30 days (Wetzel DOI: 10.7266/N7WH2NGJ). In a laboratory study using adult Gulf killifish, genotoxicity was observed in the form of single-stranded DNA

breaks, responsive genes for DNA damage, and apoptosis when exposed to weathered DWH surrogate oil WAF concentrations ranging from 300 μg/L to 3000 μg/L ∑50PAH. These exposure levels were chosen to simulate PAH concentrations found in field-collected sediments (284 to >8000 μg/L ∑50PAH) not long after the DWH spill (Pilcher et al. 2014).

29.3.8 Reproduction

Reproduction in fish is controlled by an organized endocrine system, the hypothalamic-pituitary-gonadal axis (HPG axis), which consists of the hypothalamus, pituitary gland, and gonadal glands. In oviparous females, the HPG axis is referred to as the HPG-liver axis given that many egg yolk and chorionic (e.g., vitellogenin and choriogenin) proteins are synthesized in the liver, necessary for oocyte growth and development.

There is evidence that oil exposure can impact reproductive fitness of fish through endocrine disruption and cytotoxic effects on germ cells (Iwanowicz and Blazer 2009; Zhang et al. 2016; Lee et al. 2017). However, there have been relatively few studies investigating the impacts on reproduction from the 2010 DWH oil spill. Microarray and RNA-seq analysis of gene expression in field-collected Gulf killifish showed a decrease of vitellogenin, choriogenin, and zona pellucida (glycoprotein layer surrounding egg) genes in the liver (Whitehead et al. 2012; Garcia et al. 2012) coinciding with upregulation of the CYP/AhR pathway genes. Though these studies illustrate alterations of genes involved in egg development, what remains unclear are the long-term consequences oil exposure may have on egg development and fecundity. In a full life-cycle (larvae through reproduction) study using sheepshead minnows exposed to oil-contaminated sediments for 19 weeks, Raimondo et al. (2016) were able to demonstrate a decrease in the first-generation fecundity of fish exposed to 358 and 751 mg/kg ∑50PAH contaminated sediments. While these studies may shed light on possible mechanisms of impairment, additional studies are warranted to determine the sublethal effects of the DWH oil spill on reproductive fitness and the ramifications those effects have on wild populations.

29.3.9 Transcriptomic Effects Not Yet "Anchored" by Phenotypic Observations

The use of global transcriptome profiling techniques has great utility for understanding subtle, sublethal effects of contaminant exposure on exposed species (Schirmer et al. 2010). The recent development and adoption of RNA-seq as a high throughput sequencing approach has allowed researchers to rapidly and accurately quantify the state of the transcriptome in non-model fish species following exposure

to anthropogenic and environmental stressors (Vega-Retter et al. 2018). *Genetics and oil: Transcriptomics, epigenetics and genomics as tools to understand animal responses to exposure across different time scales* (see Portnoy et al. 2020) discusses what the field of transcriptomics is and what it can tell us about effects of exposure to oil.

Several studies have linked transcriptome changes following exposure to DWH oil with endpoints of ecological significance. Whitehead et al. (2012) showed differences in gene expression profiles of Gulf killifish in response to oil contamination from the 2010 DWH oil spill, particularly decreased physiological functions in osmoregulation, respiration, and excretion, which persisted for 2 months post-oiling. Oil exposure affected multiple molecular pathways in developing mahi-mahi, including E1/E2 signaling, steroid biosynthesis, and ribosome biogenesis pathways (Xu et al. 2016). Dubansky et al. (2013) showed that Gulf killifish collected from areas affected by DWH oil had significantly altered transcriptional patterns, including a number of genes and pathways involved in xenobiotic response or oil detoxification. Exposure to very low levels (<5 μg/L∑PAH50) caused altered expression of many pathways in early life stage red drum in a manner that was dependent on both oil type and age of the embryo (Xu et al. 2017b), indicating that results from one exposure regime or age class, even within species, may not be fully informative of the transcriptional effects of other exposure regimes or ages. Exposure to oil-contaminated sediment altered liver transcriptional patterns in juvenile southern flounder, including effects on a number of immune-related genes, matching an observed oil-induced immunosuppression following bacterial challenge (Bayha et al. 2017).

29.4 Conclusions and Directions for Future Research

Although direct comparisons among species differences are scarce and further studies clearly are needed, the available literature demonstrates significant species-specific differences in sensitivity underscoring the importance of test organism choice. Similarly, life stage-dependent sensitivity differences are also obvious with early life stages generally being more susceptible. However, later life stages should not be ignored as some endpoints, including cardiac function, immune system function, and sensory and central nervous systems, may show effects even at relatively low exposure concentrations in later life stages.

In addition to choice of organisms and life stages to be tested, the endpoint examined will influence the impression of sensitivity. Cardiac development and cardiac function have long been viewed as central targets for oil exposure and are sensitive to oil exposure at low and environmentally realistic levels (<10 μg/L∑PAH50). However, recent studies have revealed that eye development and immune system function can be impacted at lower concentrations (<1 μg/L∑PAH50) illustrating the importance of considering multiple endpoints. Transcriptome wide responses have been employed successfully in recent years to shed light on additional relevant

endpoints by examining sensitive biochemical pathways and have in some cases been matched with observations of phenotypic change.

To date, the laboratory studies discussed in this chapter examining the effects of exposure to DWH oil on the immune system of GoM fish clearly show evidence of immunosuppression at the gene level, in both the innate and adaptive immune responses. Conversely, field studies from DWH oil-contaminated areas showed an activation of immune response at the gene level, but immunosuppression at the cellular level. These field studies, however, did not provide oil concentrations for these contaminated collection sites which make direct comparison to laboratory-based studies difficult. The effects of oil on fish immunity can be contradictory and will depend on many variables including the concentration of PAHs, mode of exposure, life stage, and species (Reynaud and Deschaux 2006).

The field of ecological oxidative stress research is still relatively new and assessments of select oxidative markers appear to be valuable in determining the effects of oxidative processes on the fitness of individual organisms (Beaulieu 2013). Results from these different exposure route studies suggest decreased antioxidant defenses and increased oxidative damage (via LPO) in targeted fish species when exposed to PAHs, regardless of exposure route. A review of findings on pollution in aquatic environments by Birnie-Gauvin et al. (2017) shows similar oxidative stress responses have been reported in other fish species. More controlled mechanistic studies in tandem with field studies are needed to better connect oxidative stress with PAH contaminants and understand the possible implications of oxidative stress on wild populations.

In fish, there have been numerous studies associating PAHs with reproduction disruption resulting in either sex hormone modifications (endocrine disruption) or by physiological degradation of gonads and egg development (Vignet et al. 2016). In regard to the DWH oil spill, too few studies were done assessing at reproduction; however those conducted did observe a decrease in egg development genes and in fecundity. Therefore, more studies are warranted to better understand the long-term consequences of DWH oil exposure to GoM fish.

An inherent challenge associated with impact assessments of oil spills and other catastrophic events comes from working largely at the sub-organismal level to predict impacts at the population and ecosystem levels. The focus on understanding organismal level effects can be attributed to experimental feasibility and high-resolution outcomes one can obtain in carefully controlled experiments. In contrast, studies of wild populations and their interactions are logistically challenging and most often include variables other than the environmental insult under study which can lead to confounding results. Ecosystem level impact studies are obviously of great importance and may be guided, in an effort to optimize use of resources, by careful considerations of relevant endpoints in controlled laboratory studies. Ideally, such laboratory studies will lead to testable predictions of responses by wild populations which may or, may not, be subsequently validated. The most fruitful approach to impact assessment is not either laboratory studies or field studies but rather a coordinated integrative approach where biological levels of organization ranging from molecular to integrative organismal responses through ecosystem level impacts are examined.

The use of broad mortality indices such as LC50 is appealing in the sense that they provide a discrete quantification of the effects of exposure that can be compared across species, life stages, ecosystems, and stressors. They are also easily used to calculate economic effects of contaminant releases, a necessity given the NRDA legal framework that often accompanies such a release. However, a focus on mortality indices alone can obscure the fact that real harm, at both individual and population levels, can be a result of exposure, but not manifest as overt mortality within the available or selected observation window. Harm to organisms or ecosystems that does not result in mortality is still, nonetheless, harm. Using cellular, molecular, or sublethal endpoints as discussed in this chapter can provide useful information about the potential sublethal effects of a given exposure on selected species and ecosystems than can be provided with mortality alone.

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