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SYMPOSIUM

Interactions between Oil-Spill Pollutants and Natural Stressors Can Compound Ecotoxicological Effects

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Synopsis Coastal estuaries are among the most biologically productive habitats on earth, yet are at risk from human activities including marine oil spills. The 2010 Deepwater Horizon oil spill contaminated hundreds of kilometers of coastal habitat, particularly in Louisiana's delta. Coastal estuaries are naturally dynamic habitats where periodic and stochastic fluctuations, for example in temperature, salinity, nutrients, and hypoxia, are common. Such environmental variability regularly imposes suboptimal conditions for which resident species must continually compensate by drawing on diverse physiological abilities. However, exposures to oil, in addition to their direct toxic effects, may interfere with functions that normally enable physiological compensation for suboptimal conditions. This review summarizes the panoply of naturally-encountered stressors that may interact with oil, including salinity, hypoxia, pathogens, and competition, and the mechanisms that may underlie these interactions. Combined effects of these stressors can amplify the costs of oil-exposures to organisms in the real world, and contribute to impacts on fitness, populations, and communities, that may not have been predicted from direct toxicity of hydrocarbons alone. These interactions pose challenges for accurate and realistic assessment of risks and of actual damage. To meet these challenges, environmental scientists and managers must capitalize on the latest understanding of the complexities of chemical effects of natural stressors on organisms, and adopt integrative and holistic measures of effect from the molecular to whole-animal levels, in order to anticipate, characterize, diagnose, and solve, ecotoxicological problems.

Introduction

Controlled laboratory experiments are often relied upon to characterize the sensitivity of species at risk to environmental stress in order to make assessments of damage to natural resources following environmental disasters, and for assessments of risk to the environment in anticipation of potential damage. Given estimates of the likelihood, intensity, and duration of exposures, and the types and severity of the biological responses that are anticipated, judgments are formulated to summarize or predict impact or risk. Exposures in the laboratory are also performed to establish a link between a cause (a particular stressor) and a particular effect observed in the field. Interpretation of these laboratory-based efforts can be complicated by the complexities of life in natural ecosystems.

Within the context of contaminants, laboratory-based assessments of exposure to chemicals usually are performed under optimal environmental conditions, including optimal temperature, salinity, availability of oxygen, absence of predators, and minimized competition. However, prevailing environmental conditions experienced by residents of natural systems may often be suboptimal. This is particularly true of coastal estuaries that are at risk from marine oil spills. Coastal estuaries are naturally dynamic and highly variable habitats, that are subject to much periodic and stochastic fluctuation, for example in temperature, salinity, hypoxia, and

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availability of nutrients (Wolfe 1986). Many estuarine ecosystems, including those in the northern Gulf of Mexico (nGOM) impacted by the Deepwater Horizon oil spill (DHOS), are already highly modified and impaired by human activities. Moreover, complicated interactions are anticipated between contaminants and environmental variability introduced by climatic change (Schiedek et al. 2007).

Concentrations of chemicals that might be deemed of insignificant risk based on results from experiments on responses to controlled exposures might underestimate risk in the real world, where synergistic interactions with common co-occurring natural stressors might significantly amplify the risk of chemically-induced damage (Sih et al. 2004). That is, toxicant stress may be compounded by naturally-encountered stressors, such that the nature and severity of effects may often be underestimated in ecotoxicological assessments of risk and/or damage (Heugens et al. 2001). That stressors interact is not a new or novel message, but this message must achieve greater penetrance in applied environmental biology to achieve state-of-the-art estimation of risks, and the phenomenon of interactions is one that could benefit from greater mechanistic insight.

Reviewed here are the types of environmental stressors that naturally occur in estuarine habitats, and the evidence for the nature, mechanisms, and consequences of their interactions with contaminating petroleum hydrocarbons from oil spills. In particular, interactions of oil with salinity, hypoxia, pathogens, and energetics are highlighted, including brief mention of community-level interactions that are more thoroughly reviewed elsewhere (e.g., Peterson 2001). Since it is currently the largest accidental marine oil spill in history, this review is set within the context of the Deepwater Horizon (DWH) blowout in 2010, during which approximately 200 million gallons of South Louisiana crude oil was released into the nGOM over the course of 87 days (Crone and Tolstoy 2010). This spill contaminated over 650 miles of coastal habitat centered primarily on Louisiana's sensitive delta (National Commission 2011), and unquantified tracts of deep-water habitat, where effects on resident coastal and deep-water species are starting to emerge (Silliman et al. 2012; White et al. 2012; Whitehead et al. 2012; Dubansky et al. 2013).

Salinity

Coastal estuaries are subject to wide periodic and episodic variations in salinity, due to variable discharges from rivers, marine inundation from storm surge, and regular tidal and seasonal influences. Physiological compensation for changes in salinity are a continuous challenge for resident species, since tight regulation of physiological ion balances is critical for maintaining organismal performance and fitness in many marine species. Gills are the primary organ for ion regulation (Evans et al. 2005) and these structures are in direct contact with the aquatic environment. As such, if hydrocarbon pollutants damage the epithelium of the gills this may exacerbate impacts on the health and performance of residents of osmotically dynamic habitats. Indeed, there is much evidence to support this prediction.

Some interactions between the toxicity of oil and ambient salinities may be related to changes in routes of exposure. For example, solubility of PAHs increases with decreasing salinity, thereby increasing bioavailability and toxic risk in low-salinity waters (Ramachandran et al. 2006). In contrast, fish in hyper-osmotic conditions tend to increase drinking rate, thereby potentially increasing intestinal contact with dissolved organic pollutants in seawater. Killifish (*Fundulus heteroclitus*) in seawater accumulated greater doses of naphthalene than did killifish in brackish or fresh water, and this correlated with elevated osmotic imbalance and elevated mortality in seawater (Levitan and Taylor 1979). However, killifish in freshwater also had elevated mortality relative to fish in iso-osmotic conditions (Levitan and Taylor 1979), indicating that additional mechanisms, perhaps associated with the solubility of PAHs or with general osmotic stress, can enhance PAH-induced mortality. During the DHOS event, a massive freshwater diversion on the Mississippi River (LA) was opened in an effort to limit the penetrance of contaminating oil into Louisiana marshes, and this diversion caused substantial drops in salinities in many normally brackish habitats (Bianchi et al. 2011). Such interventions can impose acute physiological stress on resident species (e.g., Eberline 2012), which alone, or in combination with other stressors such as contaminating oil, may have unintended consequences.

Some fish are facultative air breathers, providing the behavioral option of lowering the respiratory contact of the gills with oil-contaminated water. Indeed, the surface-breathing behavior of armored catfish (*Hoplosternum littorale*) increased at low to moderate concentrations of the water-accommodated fraction (WAF) of crude oil (Brauner et al. 1999). However, since fish breathing at the surface can gulp water while breathing there, they may ingest floating oil, and their occupancy of the air–water interface

may lead to direct oiling of skin and gills. Oral doses of crude oil impair osmoregulatory abilities in armored catfish (Brauner et al. 1999).

Exposure of rainbow trout (*Salmo gairdneri*) to oil–water emulsion causes impairment of osmoregulation under both hypo-osmotic and hyper-osmotic conditions, although there was no effect following IP injection (Engelhardt et al. 1981). This indicated that the osmoregulatory disruption was from direct effects on the gills rather than from systemic effects. This finding is consistent with widely reported impacts of crude oil on the morphology of the gills. The swelling of epithelial cells, as well as epithelial lifting, hyperplasia, aneurisms, proliferation of mucocytes, and lamellar fusions are examples of structural alterations of gills that commonly are induced by petroleum hydrocarbons (DiMichele and Taylor 1978; Mckeown and March 1978; Engelhardt et al. 1981; Haensly et al. 1982; Solangi and Overstreet 1982; Prasad 1991; Claireaux et al. 2004; Negreiros et al. 2011) (Fig. 1). Killifish exposed in the field to contaminating oil from the DHOS also show these structural lesions (Dubansky et al. 2013; Whitehead et al. 2012). These morphological alterations are likely causally related to changes in permeability of the gills and loss of ionoregulatory ability that have been observed in fish and in shrimp both in freshwater and seawater (e.g., Baden 1982; Kennedy and Farrell 2005; Matsuo et al. 2005; Goanvec et al. 2011). Tropical freshwater fish (tambaqui: *Colossoma macropomum*) were exposed to dispersed crude oil in freshwater, which caused ionoregulatory impairment through alterations in permeability of the gills (Duarte et al. 2010). Specifically, treatments caused diffusive loss of sodium across the gills which, when coupled with inability to stimulate sodium uptake, resulted in severe net loss of sodium. Treatments also caused severe net loss of chloride. In addition to gross morphological impacts, hydrocarbons may interact with osmoregulatory abilities by affecting sodium-potassium ATPase activity in gills (Boese et al. 1982; Mccloskey and Oris 1993) or by causing loss of chloride cells from the gills' epithelium (Goanvec et al. 2011). Recovery of the structure and function of gills may be protracted (Baden 1982; Goanvec et al. 2011). Indeed, gills from resident killifish showed much structural damage for many months following peak oiling from the DWH disaster (Whitehead et al. 2012; Dubansky et al. 2013).

Hypoxia

Hypoxia is common in estuaries because of high organic content, high nutrient levels, high temperatures, and shallow depths. Furthermore, human-induced alterations of watersheds are causing increasing severity and frequency of coastal hypoxia (Rabalais and Turner 2001; Diaz and Rosenberg

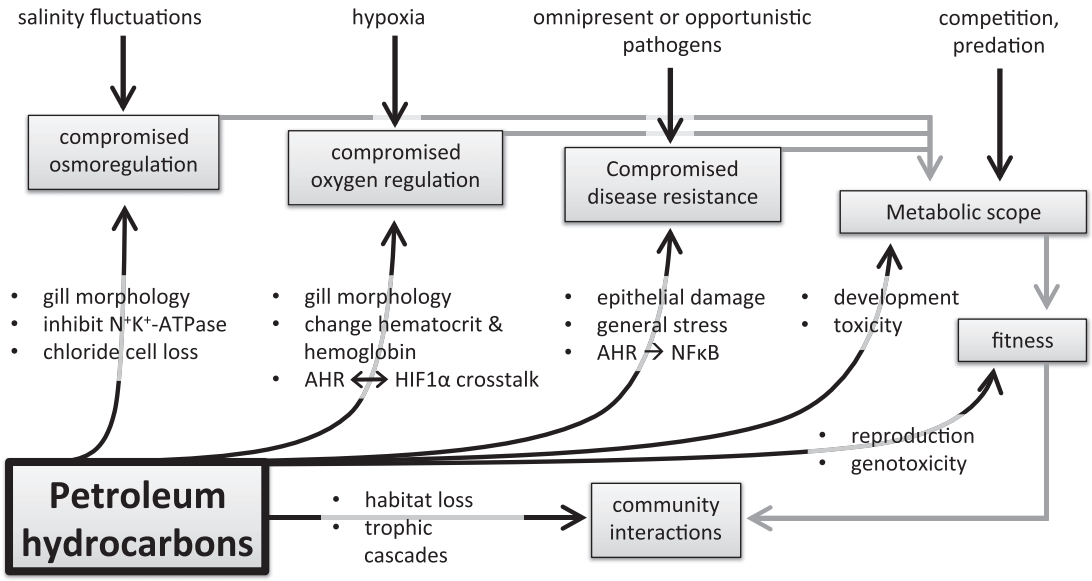

Fig. 1 Conceptual model of interactions between petroleum hydrocarbon pollutants and environmental stressors that are naturally encountered in estuaries. Natural ecological stressors (top row) are connected by black down-facing arrows to the physiological functions that are impaired by interactions with petroleum hydrocarbons. Petroleum hydrocarbons are connected to physiological effects by remaining black arrows. The mechanisms that underlie interactions between hydrocarbons and natural stressors are listed and superimposed above black arrows. Gray arrows indicate the flow of downstream effects.

2008), and these effects are expected to amplify with global climatic change. There is growing recognition that increases in the frequency, severity, and duration of hypoxia events in near-shore waters are likely to pose severe challenges for resident species, especially upon co-occurrence with contaminating hydrocarbons (Val et al. 2003). The region contaminated by spilled oil from the DWH disaster overlapped off the coast of Louisiana with one of the largest zones of marine hypoxia in the world (Turner et al. 2012).

The oil-induced structural alterations of gill epithelium that affect regulation of ions (as discussed above) are also expected to affect other functions of the gills such as gas exchange (Fig. 1). This may result in hypoxemia or compromised ability to physiologically compensate for low-oxygen (hypoxia). Furthermore, surface oil slicks may impair gas exchange at the air–water interface, thereby decreasing oxygenation of underlying waters (e.g., Malan 1986) and compounding potential challenges to the regulation of dissolved oxygen in resident animals.

Sole and Antarctic fish that were exposed to fuel experienced impaired abilities to extract oxygen at low oxygen tensions, indicating impaired compensatory ability for hypoxia (Davison et al. 1993; Claireaux et al. 2004). Exposure of flounder to WAF caused a dramatic decline in levels of oxygen in blood, which was closely followed by a release of catecholamine and a decline in hematocrit and hemoglobin of the blood (Alkindi et al. 1996). Similarly, exposure to oil caused a drop in ATP:hemoglobin levels in exposed fish, which can be indicative of hypoxemia (Brauner et al. 1999). Exposure of crabs to crude oil WAF affected respiration rates, and the oxygen tension at which death occurred was higher for crabs exposed to oil than for control crabs; thus there was an increased risk of hypoxic death (Malan 1986). Furthermore, as dissolved oxygen decreased, crabs exited their burrows and climbed through the oil slick at the surface to escape the oxygen-depleted water, thereby exposing themselves to floating crude oil and to an increased risk of direct toxic effects (Malan 1986). Exposure to fluoranthene and hypoxia had synergistic impacts on developing zebrafish, in which the co-exposure dramatically increased rates of pericardial edema and spinal deformities relative to individual effects of fluoranthene and hypoxia (Matson et al. 2008). That is, hypoxia amplifies the developmental toxicity of some PAH compounds, and also amplifies the genotoxicity of petroleum hydrocarbons (Negreiros et al. 2011). Similarly, synergistic effects on embryo toxicity in developing zebrafish were detected between hypoxia and benzo[a]pyrene, between hypoxia and benzo[k]fluoranthene, and between hypoxia and complex mixtures of PAHs (Fleming and Di Giulio 2011).

The molecular mechanisms that are responsible for physiological interactions between PAHs and hypoxia are not fully known. However, there is much molecular overlap in intracellular signaling pathways between PAHs and hypoxia (e.g., AHR signaling and HIF1α signaling) (Prasch et al. 2004; Harper et al. 2006; Matson et al. 2008; Fleming et al. 2009) that could facilitate synergistic effects between these two stressors.

Pathogens

Most wild animals are carriers of pathogens, but under normal environmental conditions tend to be asymptomatic because of a robust immune system. However, upon environmental stress the immune system may be compromised, enabling outbreaks of pathogens that can affect health and survival of hosts in the wild (Snieszko 1974). Petroleum hydrocarbons, by way of their known immunotoxicity (for review, see Reynaud and Deschaux 2006), behave as such a stressor by compromising the immune system and thereby increasing the risk of disease, and disease-associated impacts on fitness in exposed populations.

Following the Exxon Valdez oil spill (EVOS), disease was a suspected contributing factor to the collapse of the Pacific herring fishery in Prince William Sound (PWS) and its unexpectedly slow recovery. Viral hemorrhagic septicemia virus (VHSV) is indigenous in wild populations of Pacific herrings (Meyers et al. 1994). Following exposure of wild-caught Pacific herring to weathered crude oil, increasing PAH concentrations were correlated with increases in VHSV infection and in the mortality of exposed animals; infection correlated with a decrease in inflammatory cells in the liver and indicated a mechanistic link with immunosuppression (Carls et al. 1998). In 1993, four years after the EVOS, the herring fishery in PWS collapsed, and VHSV was at elevated levels in the Pacific herring that had survived (Meyers et al. 1994). Although a direct link between the EVOS and the 1993 collapse of herring is difficult to establish, indirect effects, for example through facilitating disease, cannot be ruled out (Carls et al. 2002).

Enhanced susceptibility to infection from exposure to oil could emerge indirectly from epithelial damage (e.g., alterations in the structure of the gills and their mucus linings) that compromises this physical barrier to infection, or directly through molecular

interactions of hydrocarbons with components of the immune system (Fig. 1). The intracellular mechanisms through which PAHs exert their immunotoxicity appear conserved between mammals and fish, although the types of immune effects observed depend on the type of PAH, the route of exposure, the concentration, and the time-course of response (Reynaud and Deschaux 2006; Kennedy and Farrell 2008). Intracellular mechanisms of PAH-induced immunotoxicity are associated with PAH's activation of the AHR, CYP1A1-mediated PAH metabolism, and mobilization of intracellular calcium (Reynaud and Deschaux 2006). The AHR may be a critical node that links molecular responses to exposures to PAH with dysfunction of the immune system. Many AHR agonists can cause impairment of the immune system, and emerging evidence indicates that immunity may be mediated through AHR cross-talk with TGF-β signaling and induction of regulatory T cells, and through cytokine-mediated effects of AHR on development of T helper 17 cells (Stevens et al. 2009).

Challenge by pathogens is a comprehensive test for immunotoxicity as it is a direct test of an organism's resilience and its fitness in the wild (Wester et al. 1994). Pacific herring exposed to crude oil WAF initially (at 1 day post-exposure) exhibited an increase in resistance to infection with a model bacterial pathogen (*Listonella anguillarum*), but by 4 days this resistance had disappeared (Kennedy and Farrell 2008). This initial defense could not be sustained over time; during chronic exposures (57 days) susceptibility to infection-related mortality was significantly elevated, and this effect was correlated with a collapse of the defense otherwise afforded by macrophages. Japanese flounder exposed to oil and VHSV experienced dramatically increased mortality relative to that experienced from exposures to either stressor alone (Song et al. 2011). Similarly, exposures of Japanese medaka to B[a]P caused a decrease in resistance to infection by a bacterial pathogen (*Yersinia ruckeri*) in parallel with cellular indicators of suppressed immune function (Carlson et al. 2002). These studies clearly indicate that exposure to petroleum hydrocarbons increases the risk of disease outbreaking in wildlife. Contamination by DWH oil correlated with divergent expressions of genes related to the immune system in field-collected killifish (Dubansky et al. 2013), including genes such as hemopexin, complement factor D, interleukin 8, alpha-1-acid glycoprotein, and CCAAT/enhancer-binding protein beta. Although impairment of the immune system was not directly tested in killifish exposed to DWH oil, analysis of gene pathways and gene networks of gill transcriptome responses associated with oiling implicated alteration of immune function (Dubansky et al. 2013). Given the volume of oil spilled during the DWH disaster, contamination of densely occupied habitats, documented exposures to wildlife, and the well-known immunotoxicity of PAHs, impairment of the immune system and impacts on associated fitness of resident species is likely (Barron 2012).

Energy budgets and exercise performance

Physiological energetics can provide an integrative measure of the effects of pollutants on whole organisms, in which disturbance of energy balance can link cellular/molecular effects of xenobiotics with fitness effects since deficits in energy budget often result in compromised growth, performance, reproduction, or survival (Widdows and Donkin 1991). Since compensations for other naturally-encountered stressors, such as salinity, hypoxia, temperature, or disease, are also energetically costly, the addition of stress from oil pollution can overdraw the energy budget such that resilience to native stressors is impaired (Fig. 1). Aerobic scope for activity provides a framework for interpreting how the environment affects an animal's capacity for performance (Fry 1947, 1971; Kassahn et al. 2009). Furthermore, decreases in scope for growth caused by contaminants have been linked to community-level impacts in the field, including decreases in species diversity and species richness (Crowe et al. 2004).

Exposures to petroleum hydrocarbons affect metabolic scope in diverse species including fish and invertebrates. Exposures to oiled mesocosms and oil-contaminated sediments caused reduced growth in flatfish for months after exposure (Moles and Norcross 1998; Claireaux et al. 2004). Concentrations of cellular ATP and ADP were reduced in common sole (*Solea solea*) exposed to fuel (Claireaux et al. 2004), and exposures caused a pronounced decrease in active metabolic rate compared to standard metabolic rate, resulting in significant decrease in metabolic scope. These effects correlated with morphological disruption of the epithelium of the gills and with a decrease in the contractility of heart muscle. Additional studies in sole indicated that exposures to oil decreased active metabolic rate by a greater degree than it did basal metabolic rate, thereby causing a net decrease in metabolic scope (Davoodi and Claireaux 2007). These results indicated that exposures to oil did not significantly impact the energy required for minimal metabolic maintenance, but did impair the animal's ability to

mobilize resources when challenged with elevated metabolic demand. Indeed, when additionally challenged by low oxygen stress or temperature stress, animals exposed to oil failed to compensate as well as control animals (Davoodi and Claireaux 2007; Claireaux and Davoodi 2010). PAH impacts on metabolic scope may be predictive of population-level effects in sole, as reduced recruitment was recorded for the year-two age class of resident sole 2 years after the *Erika* oil spill in the Bay of Biscay (Davoodi and Claireaux 2007). Strong correlation between increasing tissue burdens of PAHs and decline in scope for growth has also been established for bivalves both in field studies (e.g., Widdows et al. 1995; Widdows et al. 1997; Widdows et al. 2002; Toro et al. 2003) and in laboratory studies (e.g., Jeong and Cho 2007). Impacts of PAHs on metabolic scope for activity may be underpinned by compromised gills (as discussed above), in which impaired ion homeostasis causing increased osmoregulatory costs (Beamish 1978), coupled with impaired transport of oxygen, can tax the animal's energy budget and thereby narrow the scope for activity.

Performance of swimming often is considered an integrative measure of fitness for aquatic animals insofar as locomotion can directly impact success in foraging and in avoidance of predators (Webb 1986) and has been associated with competitive advantage (Castleberry and Cech 1986). Low concentrations of WAF reduced the performance of swimming by Pacific herring, and impaired their ability to recover from bursts of swimming (Kennedy and Farrell 2006). These effects were amplified by increasing concentration of WAF and increasing duration of exposure to it, and correlated with increasing osmoregulatory dysfunction and impaired response to stress. Similarly, studies in Coho salmon showed that exposure of longer-duration to WAF caused decreased swimming performance even at lower concentrations (Thomas and Rice 1987; Thomas et al. 1987), and 28-day exposures caused decreased food intake, growth, and swim performance in seabass (Gravato and Guilhermino 2009).

Exposures to PAHs during development cause well-documented developmental abnormalities in the cardiovascular system of vertebrate animals, including fish (Incardona et al. 2004). Importantly, exposures to very low concentrations of PAHs that do not cause obvious deformities in early-life stages of development, do, however, cause alterations of heart morphology and also reduce performance in swimming in adults (Hicken et al. 2011). Such morphological and physiological effects may explain the impacts of exposures to PAHs during sensitive early-life stages on fitness of animals in the wild. For example, sublethal exposures of pink salmon embryos to weathered crude oil reduced survival in the wild relative to unexposed controls (Heintz et al. 2000). It is plausible that this effect could have been mediated by impaired aerobic capacity underlain by subtle developmental abnormalities of the heart, thereby causing behavioral effects such as altered competitive interactions, foraging ability, or avoidance of predators, that can emerge at the population level (Scott and Sloman 2004). Exposure to WAF made from oil collected from the ruptured Macondo well riser caused diverse developmental abnormalities in embryonic and larval zebrafish (de Soysa et al. 2012). Exposure of developing killifish embryos to field-collected sediments oiled by the DHOS was not lethal, but induced cardiovascular effects, including depressed heart rates and increased pericardial edema (Dubansky et al. 2013). Since cardiovascular impacts on adults emerge from exposures in early life to concentrations lower than those that cause obvious effects in embryos, and since exposures to sediments collected from sites impacted by DHOS caused cardiovascular effects in embryos, it is likely that the risk of cardiovascular impairments is elevated for residents of sites contaminated with DWH oil. Elevated risk of such effects, of course, also elevates the risk of impacts on fitness.

Indirect effects through biotic interactions

Assessment of damage to natural resources and of environmental impacts often relies on the study of stressors on individual species. However, since interactions between species in nature can be strong and complex, experiments on single species are of limited use for estimating the impacts of contaminants on ecosystems, in which indirect effects may cascade through trophic or competitive interactions. That contaminants may cause such indirect effects in communities is well documented (for review, see Fleeger et al. 2003), and these indirect effects may be as significant, or more significant, than the direct effects. That oil pollution causes ecological impacts through tropic cascades has been documented for major oil spills, including the EVOS (Peterson et al. 2003).

In Louisianan coastal wetlands, oil contamination interacts with human-imposed changes in ecosystem hydrodynamics to accelerate the loss of high-quality marsh habitat that supports one of the most productive fisheries in the United States. Alterations in the natural regime of water-flow in southern Louisiana

through channelization of the Mississippi River, dredging of canals, and development, have steadily contributed to erosion and subsidence of highly productive marsh habitats, making coastal Louisianan wetlands one of the fastest disappearing landmasses on the planet (Couvillion et al. 2011). Oil contamination, by killing marsh plants, has accelerated the rate of erosion following the DHOS (Silliman et al. 2012), which through habitat loss may impact the resilience of many aquatic species (Fig. 1). This salt marsh provides crucial habitat to many commercially-important species in the nGOM, such as brown shrimp, bay anchovy, and Gulf menhaden (Chesney et al. 2000), as well as Gulf killifish which are an emerging animal model for studying impacts of the DHOS. Gulf killifish are the most abundant vertebrate in coastal Louisianan salt marshes, and killifish are top contributors to energy transfer within marshes and provide an important trophic link between marshes and open estuaries (Meredith and Lotrich 1979; Kneib and Wagner 1994; Rozas and Zimmerman 2000). Demonstrated direct effects of the DHOS on resident killifish (Whitehead et al. 2012; Dubansky et al. 2013) and marsh plants (Silliman et al. 2012) in the field, and presumably in other species that share similar habitats and exposures, help set the context for potential indirect effects from the DHOS on populations and communities.

Discussion

The acutely toxic effects that follow from direct contact with contaminating oil in the early days of a marine spill, such as from fouling of feathers and fur, are well-covered by the media and therefore well-known to the stakeholders affected by the spill, to the general public, and to the scientific community. These effects are obvious, as they unfold on the surface of the sea where observation is easy, and effects are directly attributable to causes. Sublethal effects, in contrast, often take longer to emerge, and are therefore often more difficult to link with the initiating cause. For example, PAHs decrease precursor proteins of egg yolk (Sherry et al. 2006), suppress ovarian aromatase (Patel et al. 2006), destroy primordial oocytes (Mattison et al. 1980), suppress spermatogenesis (Frouin et al. 2007), degrade receptors of sex steroids (Ohtake et al. 2007), inhibit sex steroid synthesis (Seruto et al. 2005), and prolong time to hatching (Horng et al. 2010; Dubansky et al. 2013). The consequences of such effects on reproductive fitness (Fig. 1) may take a generation or more to emerge. Similarly, other sublethal effects, such as impairment of development, growth, and performance, or increased sensitivity to pathogens, also require time to unfold and be detected. Also, low to moderate concentrations of oil can long remain in the environment, particularly in the sediments, enabling persistent exposure of resident species and slowing their expected pace of recovery (Peterson et al. 2003; Culbertson et al. 2008). Because of the time lag, and because some responses are underlain by complex mechanisms, sublethal effects are more difficult to detect, and take longer to detect, than do acute effects. Counting dead animals is easy, although field counts often are fraught with uncertainty (e.g., Williams et al. 2011). More challenging is the quantification of impacts from compromised immune systems, from impaired competitive ability due to over-taxation of energy budgets or malformed hearts, from decreased resilience to spikes in temperature, salinity, and hypoxia, or from depressed fecundity and fertility. Yet, what is clear from decades of research on oil spills, is that such sublethal effects of exposure to petroleum hydrocarbon pollutants are important for predicting long-term effects on populations (Peterson et al. 2003). Importantly, this review highlights how these sublethal effects can be amplified by the contribution of suboptimal environmental conditions that are normally encountered in natural systems, thereby elevating the ecological risks of environmental pollutants. Indeed, multiple interacting factors, including low temperatures, influx of freshwater, and pre-exposure to contaminating oil from the DHOS, are hypothesized to have contributed to unusually high number of strandings of dolphins in the nGOM in the year following the spill (Carmichael et al. 2012).

The effects on the health of animals exposed to oil are many, as are the potential interactions with additional stressors. The underlying mechanisms that facilitate these effects may in some cases be unique, but may also intersect. For example, at the morphological level, compromised gill structure may underlie many physiological impairments (Fig. 1). Gills carry out diverse functions (Evans et al. 2005), including osmoregulation, acid/base balance, and gas exchange, such that the impacts of oil on the integrity of the gills likely compromises ability of the gills to enable compensatory responses to normally-encountered environmental variation, such as salinity and hypoxia, and may provide a route of entry for pathogens. Furthermore, these impairments require energy during compensation or repair, thereby taxing the energy budget that would otherwise be spent maximizing competitive abilities, foraging success, growth rate, and reproductive investments. As

such, metabolic scope for activity represents an integrative measure of the cumulative impacts of exposures to PAHs and co-occurring stressors that is relevant for estimating impacts on fitness.

The mechanisms that underlie diverse effects of petroleum hydrocarbons may also intersect at the molecular level (Fig. 1). Much of the developmental toxicity induced by petroleum hydrocarbons is mediated through the AHR signaling pathway (Clark et al. 2010), although mechanisms independent of the AHR are also important (Incardona et al. 2005). Activated AHR signaling cross-talks with many other intracellular signaling pathways (Puga et al. 2009), such that PAH-induced signaling of AHR may interfere with diverse pathways of cell signaling and contribute to impairment of other physiological processes. Intersection of AHR signaling and HIF1α signaling at the molecular level (Prasch et al. 2004; Harper et al. 2006; Matson et al. 2008; Fleming et al. 2009) could facilitate synergistic effects between these two stressors. Furthermore, activation of AHR interferes with immune signaling through cross-talk with TGF-β signaling, induction of regulatory T cells, development of T helper 17 cells, interference with macrophages' activities, lymphocyte proliferation, and increase of intracellular calcium (Reynaud and Deschaux 2006; Stevens et al. 2009). Considering these interactions with oil at the morphological and molecular levels, environmental scientists are challenged to deploy measures of multiple endpoints, including global discovery-based "omics" tools, that cast a broad net to capture the diversity of potential biological responses.

The complexities of interactions between natural and anthropogenic stressors like oil spills highlight the need for advances in both the basic science of ecotoxicology and the practical deployment of ecotoxicology (Fig. 2). To advance the basic science of ecotoxicology, greater integration with the principles and paradigms of ecophysiology provides a path forward. Additional research is needed to distinguish how natural stressors affect the toxic impacts of chemicals by affecting each of bioavailability, toxicokinetics, or the sensitivity of organisms (Heugens et al. 2001), or combinations of those three variables. Bioavailability of PAHs and metals can be higher at low salinities by increasing their solubility. Yet, environmental regulation remains based on measurements of total concentrations of chemicals, rather than on measurements of bioavailable concentrations (Van Straalen 2003) that are more relevant for ecological physiology. Toxicokinetics may be influenced by salinity and oxygen concentrations; high salinity can increase drinking rates of fish and low oxygen can elevate ventilation rates, thereby increasing exposure to dissolved chemicals through the gut and across the gills, respectively. The toxicokinetcs of PAHs may also be modified by UV radiation; enhanced exposure to UV can dramatically increase the toxic effects of some oils (Incardona et al. 2012b), and thereby increase the risk to organisms occupying shallow habitats (Incardona et al. 2012a). Generally, it is not well-understood which of the many components of oil are responsible for which of the many toxic effects induced by oil. As oils from different parts of the world vary dramatically in their chemical makeup (Wu et al. 2012), data that link specific chemical constituents to particular endpoints of toxicity would have practical applications for estimating the risks of oil spills in different parts of the world. This may be achieved, in part, through modern advances in mixture-modeling (Backhaus and Faust 2012). Suboptimal conditions can tax the energy budget, such that less energy remains available for compensatory responses to additional stressors, thereby sensitizing organisms to pollutants. Dynamic energy budget (DEB) theory (Nisbet et al. 2000; Kooijman 2001) provides a coherent framework for describing how acquired resources are distributed and partitioned to support development, growth, and reproduction, in which toxicants can be viewed as agents that disrupt processes of allocation (Baas et al. 2010). Since advances in DEB methods have proven useful for modeling the physiological impacts of chemical mixtures (Baas et al. 2010), expansion of the models to include mixtures of chemical and natural stressors would appear a natural extension. The discussion so far in this paragraph addresses how natural stressors affect the toxicity of chemicals. The problem may also be approached from the reverse angle, in which chemical effects can sensitize the organism to natural stressors. PAHs affect the structure of the gills' epithelium, which in turn compromises physiological functions associated with ion homeostasis and gas exchange. Furthermore, molecular responses to the toxic components of oil can cross-talk, for example, with the pathways of hypoxia signaling and immune-system signaling. This interference may contribute to enhanced impacts of oil in organisms challenged with hypoxia or pathogens. Advances in research on systems biology that seeks to more explicitly link changes in gene expression to changes in ecologically-relevant characters such as growth, performance, and reproduction, are likely to enrich our abilities to predict and track the effects of multiple stressors on physiology. Such systems-biology models would be a natural complement to DEB models, and

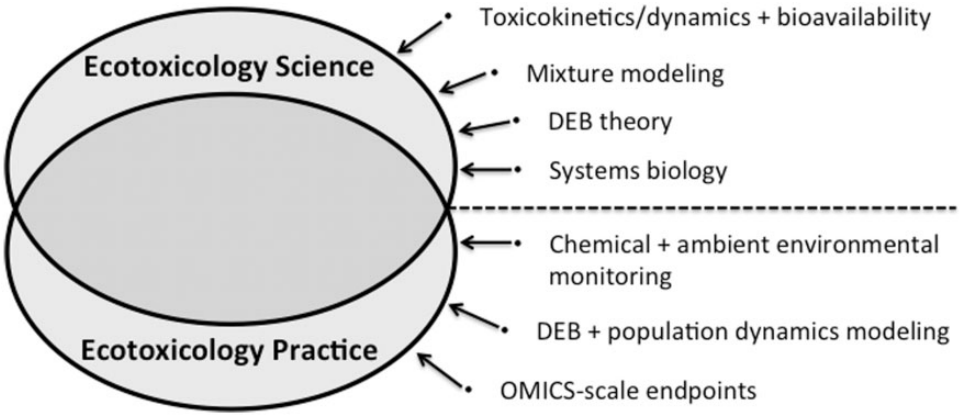

Fig. 2 Model suggesting how modern paradigms and methods may be applied to advance both the basic science and the practice of ecotoxicology, in ways that lead to greater synthesis with ecological physiology, and that lead to more accurate and useful tools for assessments of risks and damages from oil spills in natural systems. Although the objectives of the science and of the practice of ecotoxicology overlap considerably, each also has their distinct agendas that may be advanced in distinct ways. The science of ecotoxicology could be enriched by more research into how variation in the ambient environment affects the bioavailability, toxicokinetics, and toxicodynamics of pollutants. Methodological advances in mixture-modeling could help predict the toxic effects of diverse oil types. DEB theory offers a coherent and comprehensive framework for understanding the impacts of multiple stressors on the fitness of organisms. Systems biology can offer nuanced understanding of the mechanisms that underlie the interactive effects of multiple stressors. The practice of ecotoxicology could be enriched by coupling monitoring of ambient environmental variability to monitoring of chemicals following an oil spill. Those data could then contribute to DEB modeling and population dynamic modeling to more accurately estimate risk and impacts for resident species. Genome-scale monitoring tools (e.g., transcriptomics, proteomics) could be deployed in carefully-selected sentinel species to track the complexity of changes in biological responses to the environment over space and time.

integrated together these would significantly advance the state-of-the-art for ecotoxicology.

That different ecosystems vary dramatically in the nature and dynamics of indigenous natural stressors has important implications for the practice of ecotoxicology. A review of studies of multiple stressors reveals that organisms living closer to their limits of physiological tolerance may be at greater risk of impacts from additional stressors (Heugens et al. 2001), which is consistent with predictions from DEB theory. Accordingly, assessments of risks or damage, informed by DEB modeling, may be adjusted for those community members living closer to their tolerance limits. Similarly, prediction of risk may be adjusted for entire ecosystems that vary in the proportion of community members living close to physiological limits such as tropical or polar ecosystems. The factors that govern population and community dynamics of some habitats are comparatively well-studied, such as the Pacific rocky intertidal, but others, such as salt-marsh or deepwater communities in the nGOM, are arguably not as well-understood. Advances in our understanding of the ecological variables that govern population dynamics for specific species in specific habitats will offer more nuanced frameworks for estimating ecotoxicological risk and the impacts of contaminating oil.

Much environmental monitoring following an oil spill focuses on detection of oil constituents in environmental media, where the presence of chemical is considered the main determinant of risk. Recognizing that ambient environmental factors interact with oil to affect risk, environmental monitoring after an oil spill should include collection of data, including, for example, salinity, dissolved oxygen, temperature, and pathogen loads, that could be used as modifiers of chemical risk. That is, we need to ensure the collection of data on environmental variability during the aftermath of an oil spill, and incorporate that variability into models predicting amplification or alleviation of risk of damage in resident species. Monitoring of systems-level endpoints (e.g., transcriptomics, proteomics) that inform mechanism, and of integrative measurements of physiological status (e.g., metabolic scope for activity), in carefully-selected sentinel species, could enable characterization of cause-and-effect in meaningful and useful ways. Coupled with environmental chemistry and tracking of ambient environmental variability over space and time, this approach could inform more accurate models of energy balance and population

dynamics that predict risk and that characterize damage in the real world.

The challenge to environmental scientists, and to the stakeholders in assessments of risk and damage to natural resources, is to embrace complexities inherent in interactions between contaminants and other naturally-encountered stressors, and then adopt and apply state-of-the-art paradigms and tools in ways that match our more nuanced understandings of ecological physiology. Just as industry enthusiastically capitalizes on the latest advances in geology, physics, and engineering to enable recovery of energy reserves from increasingly extreme and remote environments, so too must environmental scientists and managers be compelled to capitalize on the latest understanding of the complexities of chemical effects on organisms and communities to anticipate, characterize, diagnose, and solve, ecotoxicological problems.

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