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Deepwater Horizon Oil Spill as a Case Study for Interdisciplinary Cooperation within Developmental Biology, Environmental Sciences and Physiology

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Abstract

The Deepwater Horizon Oil Spill in the USA's Gulf of Mexico created a high degree of exposure of marine organisms to toxic polyaromatic hydrocarbons (PAHs) present in crude oil. To determine the ecological and physiological effects of crude oil on the Gulf of Mexico ecosystem, the Gulf of Mexico Research Initiative created several research consortia to address overreaching questions concerning the biological impacts of the ecology of the Gulf of Mexico that would otherwise be beyond the capabilities of an individual investigator or a small group. One of these consortia, highlighted in this article, is the RECOVER Consortium, which brings together physiologists, developmental biologists, toxicologists and other life scientists to focus on the multifaceted physiological effects of PAHs, especially as they pertain to cardiovascular and metabolic physiology of economically important fish species. Using the Recover Consortium's interdisciplinary approach to revealing the biological impacts of the Deepwater Horizon Oil Spill as a case study, we make the argument for interdisciplinary teams that bring together scientists with different specialties as an efficient way—and perhaps the only way—to unravel highly complex biological effects of marine oil spills.

Keywords

Deepwater Horizon Oil Spill, Gulf of Mexico, Interdisciplinarity, Research Consortium

1. Introduction

For decades biologists have investigated the ecological and physiological effects of oil spills in marine environments [1] [2]. In this article we highlight the collaborative scientific approach developed in response to the Deepwater Horizon Oil Spill into the Gulf of Mexico in 2010. Specifically, we draw examples from the RECOVER consortium as a case study, supported by the Gulf of Mexico Research Initiative, to show the strengths of interdisciplinary collaboration not only for this applied research in pollution-related research, but

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beyond to basic biology.

2. The Deepwater Horizon Oil Spill

2.1. The Event

The Deepwater Horizon oil well failed on April 20, 2010, killing 11 workers and causing the largest marine spill in history, twenty times the size of the Exxon Valdez spill in Alaska in 1989 [3]. In addition to this human tragedy, oil flowed from the Deepwater Horizon wellhead for 87 days during the summer. Estimates report that the Deepwater Horizon Oil Spill (DHOS) released millions of liters of oil and natural gas, fouling the landscape of the Gulf of Mexico and its shoreline [4] [5].

The oil from the DHOS is a light crude oil containing a high percentage of low molecular weight polycyclic aromatic hydrocarbons (PAHs) [6]. Polycyclic aromatic hydrocarbons are toxicants derived from crude oil, incomplete combustion of fossil fuels and other anthropogenic sources. They are ubiquitous in the environment, such that a number of the PAHs in crude oil are considered priority pollutants by the World Health Organization [7]-[9]. These more toxic PAHs are often studied singularly or in combination with other chemicals. However, crude oil is composed of hundreds of chemicals and contains dozens of PAHs that are toxic to wildlife and humans [10].

In addition to the inherent chemical complexity of the released crude oil, another complicating factor is the dynamic chemical composition of oil due to the volatility of low molecular weight PAHs. Mechanical forces (*i.e.* wave action), microbial degredation, pressure, photooxidation, and temperature are among factors that can change the composition of oil over time [6] [11] [12]. This differential loss of various chemicals from crude oil is called “weathering”, and results in varied composition of toxicants in DHOS crude oil dependent on the degree and duration of environmental exposure [6] [12]. Regardless of oil composition and weathering, PAHs are persistent, remaining in weathered oil and oil-laden sediments, potentially for decades [3] [13].

2.2. Biological Consequences of the Deepwater Horizon Oil Spill

Oil released from the DHOS traveled mostly north across the Gulf of Mexico during the summer of 2010, making landfall along 1040 km of shoreline in Louisiana, Mississippi and Alabama [14]. This time period coincided with the breeding season for numerous resident and transient species, so it is likely that breeding animals and their progeny were exposed to PAHs from DHOS crude oil. In addition to the obvious effects of immediate oil fouling, the chemicals within crude oil, particularly PAHs, can have highly detrimental effects on marine life. It is beyond the scope of this article to detail these effects, but suffice it to say that PAHs and the by-products of their biotransformation exert numerous adverse health effects that span the gamut of physiological processes including blood disorders, cardiac dysfunction, genotoxicity, epigenetic remodeling, immunodeficiency, cancer, and a wide spectrum of developmental defects. These effects can occur not only in marine and estuarine wildlife, but can also in humans exposed to PAHs [10] [15]-[22].

Exposed organisms are not without defense, as some species, depending on stage of development, are able to efficiently metabolize much of the PAHs from crude oil. The metabolism of PAHs is unified around the aryl-hydrocarbon receptor (AhR) pathway, the most studied biochemical pathway in toxicology for more than three decades [23] [24]. The lipophilic nature of PAHs enables them to freely enter the cell, where they are ligands for the AhR, a cytosolic receptor. Following binding, the ligand-bound AhR translocates to the nucleus and evokes the transcription of a suite of genes that aid in the metabolism of PAHs through biotransformation and elimination from the cell [25] [26].

Despite the well characterized biochemical activity of PAH metabolism in the cell, there are critical unknowns concerning the key alterations in gene expression and downstream alterations in physiology and morphology that govern the effects of PAH toxicity. Thus the mechanisms of action that result in physiological and morphological defects in adult and developing organisms aren’t well understood [25], nor can they be understood by a strictly cellular or molecular approach. Surprisingly, there are but a handful of published studies thus far that have examined the biological effects of DHOS oil on vertebrate species in the Gulf of Mexico, with only a small portion of those focused on physiological function and overall health effects. This represents an alarming gap in knowledge of the effects of the DHOS on the ecosystem at large.

Against this backdrop, it is clear that the DHOS disaster presents an opportunity to further our knowledge of

how a broad range of taxa respond to the effects of PAHs in the environment, and as a byproduct, further general knowledge of the effects of these ubiquitous environmental contaminants. Importantly, however, we advocate increasingly interdisciplinary approaches to advancing this knowledge. Why? Individual investigators generally assemble a series of elegant studies over many years, to represent a significant and impactful body of work, illustrating the steady but sometimes slow progression of results that is inherent in biological research. Through interdisciplinary collaboration, the results of research activities can often be more than the sum of the individual parts. It follows, that the greatest impact on the collective knowledge of the DHOS disaster, or many noteworthy problems in science, is intensive collaboration.

3. The Gulf of Mexico Research Initiative's RECOVER Consortium

Carefully planned and focused research is occurring by thousands of scientists that engage in multidisciplinary research to address gaps in knowledge, and to chart the recovery of the Gulf of Mexico ecosystem affected by the DHOS (see below). It is in this spirit of collaboration and sharing of knowledge, where unprecedented advancement can be made.

In a rapid response to the Deep Horizon Oil Spill and its consequences to the health of the Gulf of Mexico, BP announced the commitment of up to $500 million over a 10 year period to provide the funding for the establishment of an independent research program to investigate the impact of the oil spill on both the environment and public health in the Gulf of Mexico. Called the Gulf of Mexico Research Initiative (GoMRI), this research institute has funded research grants in support of both individual PIs and consortia of researchers. As drawn from the Initiative's mission statement,

" ….(*GoMRI*) *will investigate the impacts of the oil, dispersed oil, and dispersant on the ecosystems of the Gulf of Mexico and affected coastal States in a broad context of improving fundamental understanding of the dynamics of such events and their environmental stresses and public health implications. The GoMRI will also develop improved spill mitigation, oil and gas detection, characterization and remediation technologies.*"

One of the research consortia funded by GoMRI is the Consortium involving Relationships of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER) (https://www.miami.edu/recover), involving four American universities (the University of Miami, the University of North Texas, the University of California Riverside, and the University of Texas Austin Marine Research Institute). Drawing together scientists experienced in ichthyology, environmental biology, comparative physiology, toxicology, developmental biology, molecular biology, genomics, marine chemistry, fisheries and aquaculture, this Consortium brings to bear different but equally important strengths that center on the cardiovascular developmental effects caused by DHOS oil exposure in economically and ecologically important fish species such as the mahi mahi, *Coryphaena hippurus* and the red drum, *Sciaenops ocellatus*. Importantly, by following "the 3 Cs" of effective research groups —Communication, Collaboration and Cooperation—this research consortium is focusing on the critical nexus between Development, Physiology and the Environment. Equally important, although the backgrounds of the Consortium members are diverse, the *focus* of the Consortium is anything but diverse. Indeed, the collective efforts are directed towards what is believed to be the pivotal role of the cardiovascular system in the ability of fishes to survive, if not thrive in the face of anthropogenic environmental challenges.

4. Activities of the RECOVER Consortium

The research activities of the RECOVER consortium emphasize the effects of early life stage exposure to DHOS oil on the cardiovascular system of fishes *as they grow and develop*. As such, the Consortium and their collaborators are engaging in a changing paradigm in ecotoxicology, where sublethal physiological effects are being studied in lieu of the traditional mortality-based studies generally used in toxicology [1] [18] [27]-[30]. In this article, we highlight five of these activities, with the specific goal of indicating how a multi-faceted approach can make significant contributions to understanding the biological consequences of the Deepwater Horizon Oil Spill.

4.1. Understanding PAH Resistance in Fishes

One of the key information gaps to date is how animals mitigate PAH exposure at the cellular and molecular levels, as described above. In this context, the RECOVER Consortium is utilizing vertebrate species from the Gulf

of Mexico to understand how the DHOS affects resident organisms in their unique environments, where secondary stressors may differentially affect physiological function. Indeed, even intraspecific differences in the response to environmental stressors exist, and likely influence the response to crude oil [31]. Killifish populations on the East Coast of the US have variable responses to thermal and salinity stress [32] [33], and some have developed a resistance to PAH teratogenicity, and toxicity in adults [34] [35]. RECOVER physiologists and collaborators in the ecological and molecular sciences are using populations of *Fundulus grandis* found along the Gulf of Mexico coast to better understand how a species of fish may react differently to PAH exposure from oil based on their respective exposure histories. In this vein, unique Gulf of Mexico populations of *F. grandis* exhibit recalcitrant AhR activity during exposure to PAHs and are resistant to the otherwise cardiotoxic developmental defects caused by PAHs [26]. This Gulf of Mexico resident species is being utilized extensivly to determine the endogenous and induced role of the AhR pathway in the cardiotoxicity of PAHs, in studies comparing the molecular and physiological response between multiple resistant and reference populations of *F. grandis* [26] [36]. These studies suggest that even populations of a single species can respond differently to toxicant exposure and that they may also be physiologically distinct (**Figure 1**), indicating that field studies should not only contain multiple sources of experimental animals, but should also contain multiple reference populations to act as controls [36]. Further, since early life stage fish were exposed to oil during the summer of 2010 [18], additional Gulf of Mexico fish species such as redfish, mahi mahi, and cobia are being exposed to sublethal concentrations of DHOS oil at early life stages, and then being tracked through their life cycle to determine effects of early exposure on later life stage fitness and general health [22].

Against this backdrop, the primary emphasis of the RECOVER Consortium in this regard is to understand the effects of early exposure on cardiac function, a metric of the organism-level effect, potentially universal to all vertebrates exposed to PAHs, and where little understanding has been gained despite considerable past efforts [21] [37]. Using a highly collaborative approach where numerous investigators are combining resources, knowledge, unique perspectives and skills; the RECOVER consortium is leveraging a compendium of assets to pair environmentally relevant exposure regimes with fine-scale measures of physiological function and their underlying mechanisms. Diverging from traditional toxicological studies of acute mortality, in lieu of integrative measures of organismal health, this approach is implicit in its need for multiple investigators focused on various

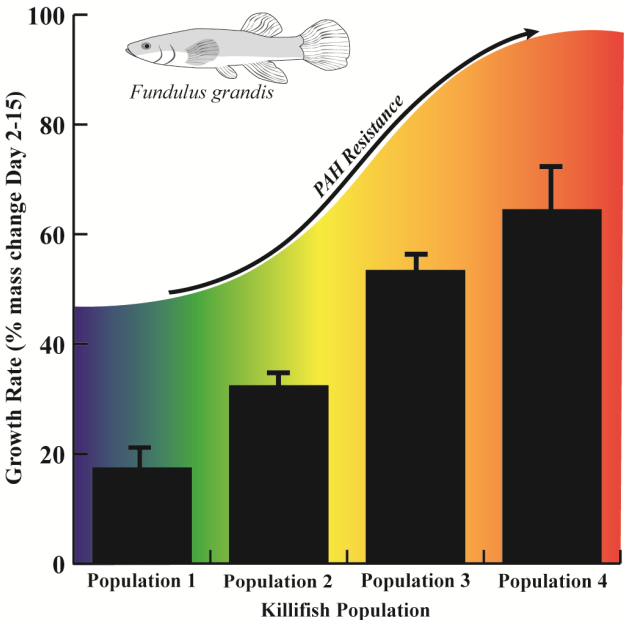

Figure 1. Distinct populations of Gulf killifish (*Fundulus grandis*) have different growth rates from day 2 to day 15, indicating that the metabolic physiology of different populations may be distinct during early life stages. Acquired resistance to PAHs and other toxicants in *F. grandis* populations may also be linked with such uniquely altered physiological function (B. Dubansky, unpubl.)

facets of cardiovascular development and toxicology. Also implicit in this *modus operendi* is the use of sublethal exposure regimes that are aimed at gaining considerable insight into the long-term cardiac (and general) effects as a consequence of early life stage exposure to DHOS oil.

4.2. Synergistic Effects of Natural and Anthropogenic Stressors

The DHOS released toxic hydrocarbons into the Gulf of Mexico, but this body of water was hardly pristine before that event, containing a variety of other categories of pollutants originating from anthropogenic sources. Laboratory approaches to studying the effects of DHOS related pollutants often involve the time-honored approach of exposing organisms to carefully regulated conditions where the only potentially disruptive component is the pollutant (or category of pollutants) being studied [38]. Yet, experimental protocols rarely involve organisms exposed to only a single stressor in their natural habitat. In fact, organisms are often experiencing environmental challenges on multiple fronts, ranging from predation and competition to temperature and oxygen fluctuations [31]. However, assessments of the potential impacts of chemical contaminants like the PAHs from the DHOS often focus on only that suite of chemicals and disregard other environmental stressors. As just one example of an additional stressor, the Gulf of Mexico, like many coastal systems into which large rivers empty, contains a significant area of hypoxic (low oxygen) waters sometimes referred to as a "Dead Zone" [39].

Why is recognition of concurrent multiple stressors of significance to determine the specific effects of DHOS PAHs? Consider as an example the fact that environmental oxygen levels have interactive effects with numerous contaminants including PAHs [40] [41]. It has been demonstrated that hypoxic environments appear to alter endocrine signaling and associated gene expression pathways, disrupting sex ratios and reproductive output potential [42]. Although it is known that AhR signaling can be modulated by hypoxia, little specific information is available regarding the mechanisms by which hypoxia may increase the potential impacts of oil on aquatic biota.

Another environmental stressor specifically relevant to the release of crude oil into the Gulf of Mexico is ultraviolet radiation (UVR) found in sunlight. Due to its location in subtropical latitudes, the Gulf of Mexico receives high levels of ambient UVR [43]. Although coastal and nearshore systems may vary widely, offshore open water systems are generally highly transparent to sunlight and UVR may penetrate in a wavelength dependent manner as deep as 30+ m [44]. Life stages of a variety of species including pelagic fish and zooplankton can be found within this photic zone in the water column, and, thus have developed numerous strategies for ameliorating the effects of UVR exposure. These include the accumulation of protective pigments from dietary sources, rapid onset of melanin pigments, and behavioral strategies such as diurnal vertical migration [45]-[49].

GoMRI's RECOVER Consortium with its toxicologists and physiologists is well poised to investigate the synergies between PAHs and UV light on larval fishes. Numerous studies have shown that co-exposure to UVR (particularly in the UV-A spectrum) and certain PAHs, including those found in crude oil, results in synergistic toxicity [50]. This phenomenon, called photo-induced or photo-enhanced toxicity, occurs when UVR light causes an increase the toxicity of PAHs compared to non-UV exposures. UVR light can enhance toxicity of PAHs by more than 100× [50]. Photo-induced addition to the toxicity of PAHs can refer to two classes of mechanisms-photosensitizers, and photomodified toxicants. A photosensitizer is a molecule that, after cellular uptake, can cause damage to cell membranes, enzymes, organelles, and DNA by generation of reactive oxygen species (ROS) in the presence of UV light of a specific wavelength range. A photomodified compound is one that has been altered in structure by UV light, enhancing its toxicity relative to the parent compound. Oxidative stress generated by these reactions can cause damage on a tissue level resulting in reduced organ function such as increased oxygen diffusion distance and reduced osmoregulation in the gill [51] [52]. Damage by an intermediary oxidation step or by direct oxidative damage to DNA can lead to DNA adduct formation, and single strand breaks [53]. PAH carcinogenicity can be enhanced by photo-activation [53]. Effects that have been reported in the literature other than increased mortality include: reduction in organism fecundity [54], increased photo-avoidance behaviors [55]-[57] and feeding inhibition [57].

The potential for PAH photo-induced toxicity to occur following an oil spill is dependent on several factors; most prominently the proportion of phototoxic PAH present in the oil, the bioavailability of those PAHs, and the intensity/duration of the UVR the organism is exposed to. Because these factors can be influenced by a variety of environmental and physiological variables, PAH photo-induced toxicity is excellent example of how an interdisciplinary team of toxicologists, developmental biologists and chemists can more readily address a given problem. For example, UVR exposure can be mediated not only by an organism's position in the water column

but also by water column characteristics such as suspended solids and dissolved organic carbon [58] [59]. It has been postulated that salinity affects sensitivity to photo-induced toxicity due to species differences in osmoregulatory strategy [60]. Oxygen diffusion distance in the gills increases likely due to edema and swelling following an exposure to PAH and UVR [52]. To understand this relationship of effects requires a combination of multiple skill sets including toxicology, histology, physiology, cellular biology, chemistry and physics that provides a more in depth understanding of the mechanisms and long-term consequences of photo-induced PAH toxicity. The RECOVER Consortium provides just such an opportunity in a cohesive unit. In previous work, we have shown that several native Gulf of Mexico species, including but not limited to early life stage crabs, mahi-mahi, and red drum are all sensitive to photo-induced PAH toxicity at concentrations well within the range observed during the DHOS (**Figure 2**) [61]. Funding provided by GoMRI will allow the multidisciplinary RECOVER team to continue to efficiently explore the mechanisms behind reduced survival of embryonic and larval stages of these species as well as potential mechanisms and outcomes of sublethal responses.

4.3. Developmental Biological Approaches to Pollution Studies

To the biologist and layperson alike, the visual effects of oil pollution on aquatic life, marine mammals and birds and the habitats they live in are often memorable and compelling. As rapid attempts at remediation occur at "ground zero", oil-fouled organisms are most evident in the adult species affected, obvious from the presence of carcasses on the shoreline [62] [63]. The presence of oil fouling the environment and the shoreline casualties invariably points to negative impacts on the reproductive success of wild populations from the loss or reduced fitness of adults. It follows that investigation of reproductive potential of affected species a key part of an effective response to oil-based pollution.

Unfortunately, an effective rescue of oil-fouled adult animals, coupled with the (re) appearance of adult wild stock in the aftermath of an oil spill, may belie the longer-term damaging changes in population that remain under the water. Field-based collection of physiological data from adult organisms may be informative [28], although it only shows a snapshot in time from each animal collected. Indeed, a more insidious effect that is ultimately as catastrophic as the effects on adult animals is the failure of embryos and larvae to develop normal morphologies, physiologies and behaviors. Such alterations in developing organisms will effectively eliminate their potential to reach reproductive age. As such, the modified environment may act via developmental phenotypic plasticity, producing maladaptive traits that result in a decline of wild populations. This impact may be rendered "invisible" because of the failure of the very smallest life cycles to grow to larger, more visible stages.

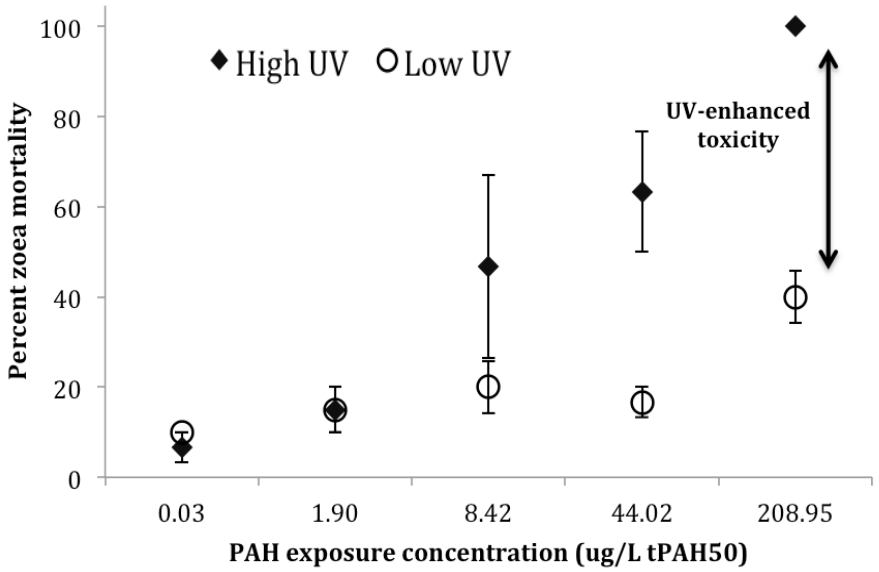

Figure 2. Mean percent mortality (±1 SE) in blue crab zoea exposed to five concentrations of naturally weathered oil and two UV treatments. The arrow indicates the photo-induced or photo-enhanced effect on toxicity. Adapted from [61].

Amongst toxicologist, ecologists and physiologists, it is widely appreciated that crude oil is particularly toxic to early life stages resulting in reduced fitness and increased mortality in exposed animals [18] [28] [64]-[67]. Breeding strategies are in accordance with maintaining a steady-state population, insuring that each individual will reproduce to create, on average, one reproducing adult [68]. It follows that any reduction in the fitness of the brood will decrease the potential numbers of future adults in a population [69]. However, it is also likely that compensatory mechanisms are in place that will offset an incident, such as the DHOS, whereas populations will recover or remain stable [70]. Weighing both scenarios, it is important to monitor the potential for population-level impacts of reduced fitness, and ultimately to understand any compensatory physiological mechanisms that may allow populations of animals to mitigate reduced breeding success.

Much data collected on the effects of DHOS on organismal health are from laboratory studies that attempt to predict the potential impact on populations and from collection and estimation of mortalities immediately following the event [62] [63]. Early life stages are particularly susceptible to stressors and widespread reduction of fitness and survival likely lead to population-level impacts although such effects are difficult to monitor in the ecosystem [69]. As such, considerable efforts by the RECOVER consortium and others are being directed at assessing the effects of exposure to DHOS oil during the embryo stage of Gulf of Mexico species *and* both model organsisms [18] [21] [22] [64] [71] [72]. As researchers move forward to assess the recovery of the Gulf of Mexico ecosystem, it will be particularly important to focus on the population characteristics that may influence the propagation of a species. Mechanisms of heredity, co-occurring stressors and the changing environment are examples of factors playing important roles, influencing future generations of DHOS oil- exposed organisms. Such considerations surely require the integration of multiple fields of expertise to gain a holistic understanding of the effects of the DHOS on the Gulf of Mexico [73].

Specifically, the developmental physiologists of the RECOVER Consortium are answering several key questions that emerge when viewing the effects of pollution through a developmental biology lens. For example, why are the early developmental stages so sensitive to environmental stressors like PAHs? Are the morphologies and physiologies of the early life stages similarly affected by PAHs? And why have they received relatively little attention compared to effects on adults of the same species?

Answering the first question is straight-forward-the early life stages are especially vulnerable because of the specific periods in development, known variously as “sensitive periods” or “critical windows”, when developing tissues, organs and organ systems may be exquisitely sensitive to disruption by stressors (e.g. [74]-[80]). Thus, a stressor that may have no effect before or after the critical period can wreak havoc on development if it appears *during* the critical window. The critical windows for many aspects of development occur within the first days of the life cycle in many fish species [81]-[83]. Indeed, a stressor that may be deleterious or even lethal in embryos and larval fishes may be easily shrugged off by the adult.

With respect to the second question, the broad answer is that physiology generally derives from morphology, so modified morphologies as a result of pollutant exposure in larval stages are also very likely to affect physiological processes. The caveat here is that modifications in morphology (e.g. reduced surface area of the gills) can be completely mitigated by changes in physiological rates (e.g., increased ventilation and perfusion of the gills), but of course at a potential energetic cost. This example highlights that changes in physiology can take the form of “simple” rate changes, or they can comprise more complex changes involving qualitative as well as quantitative alterations.

The third question posed above, namely why experiments-especially in physiology-have focused primarily on adult fishes, is underscored by a recent search of PubMed’s data base of 23 million biomedical papers. A September 2015 search for titles/abstracts containing the three words “pollution”, “physiology” plus “fish” yielded 2,538 “hits”, while a more directed, developmentally-focused searches of “pollution”, “physiology” plus either “fish *embryo*” or “fish *larva*” yielded only 108 and 58 hits, respectively. One reason why embryonic and larval fishes are studied less often than adults in pollution/toxicology related investigations involving physiological changes is that they are obviously very small! Yet, this apparent challenge to measurement on embryos and larvae is based more in perception than fact. Indeed, stroke volume, heart rate, cardiac output, intraventricular blood pressure, arterial and venous vasoconstriction and vasodilation, blood velocity and numerous other physiological parameters can be dynamically recorded in fish larva weighing as little as 1 mg using both optical observation, electrophysiological techniques and invasive micro-instrumentation ([84]-[92]). As mentioned above, there is a paucity of data concerning the effects of the DHOS on Gulf of Mexico vertebrate species. Even less is known about the effects on the developmental biology of these species, which is a key component of under-

standing the reproductive potential of populations.

Numerous additional questions under scrutiny by RECOVER include: To what degree do the effects seen in developing fish populations affect the population at large? What are the key developmental processes that are affected by PAHs that actually cause the defects? How can subtle effects that translate to decreases in fitness later in life be detected? What physiological traits enable animals to evoke compensatory responses to PAHs during development, and what tradeoffs may occur during a "successful" response to PAHs (e.g. hypoxia tolerance)?

Precisely because many of the physiological processes of fishes may be altered by toxicants, especially in the larval stages, the RECOVER consortium is answering these questions by focusing specifically on the cardiovascular and metabolic physiology of economically important fish species in the Gulf of Mexico. Even within what would seem to some to be a narrow focus, this interdisciplinary consortium involves carefully coordinated studies to determine how the pollution-derived modifications of cardiovascular physiology may threaten these key fish stocks. Thus, the RECOVER consortium is exploring the physiological underpinnings of how developing fishes cope physiologically with polluted environments, as this may provide as many, if not more, insights than limiting studies to adult fishes.

4.4. The Next Generation(s): Transgenerational Epigenetic Inheritance and Environmental Pollutants

There is an expanding interest in the intragenerational phenotypic changes (epigenetic inheritance) in fishes and other animals caused by environmental pollutants, though we are unaware of any studies involving species impacted by the DHOS. PAHs do, in fact, cause epigenetic phenotypic modification through DNA methylation, histone modification and microRNA silencing ([93]-[100]. Despite the growing interest in epigenetic inheritance, transgenerational transfer of modified phenotypes from the parental generation exposed to PAHs to their offspring has been investigated in few studies ([101]-[103]. Indeed, tractable fish models (e.g. zebrafish, medaka) are often used to study environmental stressors (e.g. [101] [104] [105]) in lieu of fish species of actual economic importance.

One interesting aspect of the emerging data sets is the complex nature of the interactions between stressor dose and generation. Consider the transgenerational effects of benzo-a-pyrene on survival and development in zebrafish larvae [105]. An elevated incidence of body morphology and craniofacial structure deformities as well as edema (a common indicator of physiological anomalies in larval fishes) occurs in not just the F1 generation, but in some cases through to the F3 generation. Moreover, some of these multigenerational effects can be either "washed in" (not appearing until the F2 generation) or "washed out" by slowly dissipating over 2 - 3 generations rather than suddenly disappearing (**Figure 3**). These findings lend empirical evidence for predicted complex epigenetic dynamics ([106] [107]).

The life scientists of the RECOVER Consortium have been exploring different facets of transgenerational inheritance in model species such as zebrafish [108], and exploring the theoretical implications of epigenetic effects [109] [110] and they are now turning attention to how PAHs may affect epigenetic inheritance of metabolic and cardiovascular phenotypes. How might knowledge e of these potential multigenerational effects of PAHs and other pollutants alter our assessment of, and response to, the impacts of oil spills on fisheries stocks? One obvious modification to our monitoring of modified phenotypes is the need to continue beyond the F1 generation, perhaps into the F2 and F3 generations. For monitoring the totality of pollution effects in Gulf of Mexico marine fish species that live several years (e.g. Mahi mahi with a 4 - 7 year life span), this can argue for a monitoring program that can literally cover decades to fully determine the transgenerational impact of epigenetic inheritance of modified morphological and physiological phenotypes.

Another implication is that an observation of "no effect" in the F1 generation means precisely that-literally there is no effect in the F1 generation. However, this does not mean that there has been no impact on the fish populations and that as a consequence, monitoring agencies can relax, because the effects may not show up until the F2 or later (**Figure 3**)!

A final implication of emerging studies is that disrupted molecular phenotypes do not necessarily translate into disrupted morphological phenotypes, and further, that modified morphological phenotypes do not necessarily translate into disrupted physiological phenotypes- and vice versa! Thus, an accurate assessment of epigenetic inheritance of modified phenotypes is really best done on multiple organizational levels within an organism,

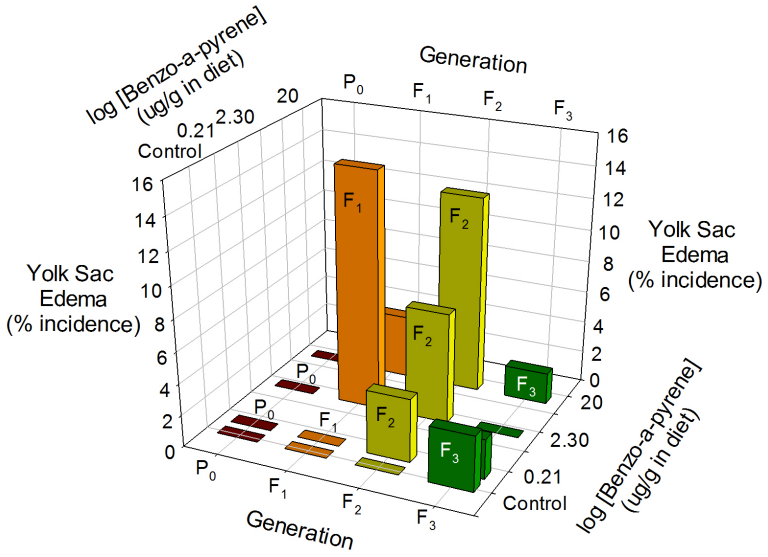

Figure 3. Transgenerational inheritance of morphological disruption (yolk sac edema) across three generations of zebrafish (*Danio rerio*) in response to dietary exposure to benzo-a-pyrene. Note that the extent of edema reflects a complex interaction of dose and generation, with edema "washing in" over two generations (e.g. 20 µg/g diet) and some "washing out" over subsequent generations (e.g. 2.3 µg/g diet). (Original data replotted from [105]).

linking molecular phenotype to physiological effect. Here, we propose that that the DHOS may represent a large scale scenario, where population dynamics may be affected simply by the "memory" of previous generations' exposure.

4.5. Exploiting Alternative Model Organisms to Understand Environmental Stressors and Their Effects

In the drive to determine the effects of pollutants on affected ecosystems, it is not surprising that life scientists typically examine those organisms most directly affected-*i.e*. those species that have shown immediate mortality or fitness declines as a result of a pollution event. In many cases, the targets for urgent and extensive investigation are the most "visible" affected species-e.g. fishes washed up on a beach or mud flat, or economically exploited species that are in apparent decline based on the development of unfilled fisheries quotas. In the case of the RECOVER Consortium, the primary foci are the mahi mahi, *Coryphaena hippurus*, the red drum, *Sciaenops ocellatus* and the cobia, *Rachycentron canadum* and other economically important species [111]-[114]. Yet, the shortest distance between two points (a particular pollutant on one hand, and on the other a comprehensive understanding of its overall effect on animals and plants within the ecosystem) may not be a straight line. **Figure 4** shows a flow chart of possible funding and research reactions to a pollution event. Often, research funding and efforts are directed towards study of the species that have been directly affected by a pollution event. This is not surprising since the DHOS provided a natural experiment of sorts, where comparison between organisms inhabiting oiled vs. unoiled sites could be conducted [18] [28]. While a useful outcome of this pathway that uses directly affected species is an understanding of the conditional characteristics of the event, this approach may create only a narrow understanding of how a class of pollutants affects a particular taxa of animals. In addition, the basic biology that transcends taxa may be obscured.

An alternative approach is to include tractable animal models that, while perhaps not the direct targets of pollution events, are highly amenable to particular experimental approaches. The use of animal models has been widely promoted (for an entry into the voluminous literature see [88] [115]-[119]), and it is not the purpose of this article to belabor this point. However, representative of the RECOVER consortium and in the theme of interdisciplinary collaboration, it is clear that toxicologists, fisheries biologists and others may benefit from collaborations with comparative physiologists. Comparative physiologists, as their name suggests, *compare* species.

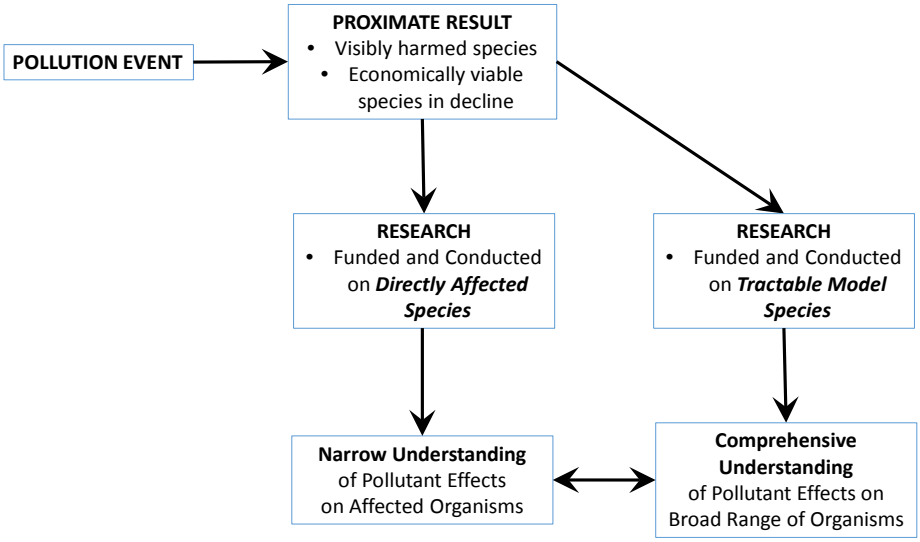

Figure 4. A conceptual flow chart of combining conventional and alternative pathways for investigating pollution effects.

They do this in an attempt to use a *few* species to understand fundamental characteristics of *all* species, not just a particular species of interest. Arising from the comparative approach is a willingness to exploit both traditional animal models but also animal animals that have particular characteristics that make them particularly tractable to experimentation. Indeed, this approach is embodied in the so-called Krogh Principle, named after the famous comparative physiologist August Krogh. Essentially, the Krogh Principle espouses that for every biological question, there is an ideal animal with which to answer it [88] [120] [121]. In the context of developmental biology, as an example, comparative developmental physiologists have over the years turned to various animal models that have various combinations of high fecundity, transparent and large embryos, or otherwise lend themselves to experimentation or observation. By using the Krogh principle to explore perhaps less obvious but otherwise more tractable avenues of experimentation, and comparing the results with what can be determined on what might actually be the species of interest, we posit that a more comprehensive overall understanding of a pollutant on the broader ecosystem can emerge (**Figure 4**).

Comparative physiologists essentially conduct "feasibility studies", exploiting a species which may not be "in the line of fire" to nonetheless flush out broader biological principles. Collaboration with comparative physiologists, even for feasibility studies, can thus render the experimental approaches of toxicologists, ecologists, fisheries biologists, etc. even more productive and efficient.

In the context of fisheries biology and aquaculture, this can mean investing time and research funding into animal models that may, on the surface, seem to have little to do with maintaining commercially important fish stocks. However, as we have noted, the convergence of countless biological pathways and traits exists in all organisms, illustrated in the commonality of the vertebrate response to PAHs. While the RECOVER Consortium is focused on studies involving the effects of DHOS oil on mahi-mahi and red drum, we are also taking advantage of tools and approaches that are conveniently available in species that can be used as models, such as killifish and even zebrafish to further understand potential oil effects on pelagic fishes. Indeed, the earlier in development that one focuses, the more sense this comparative approach makes, since essentially all developing vertebrates follow a highly conserved developmental plan evident in the early stages when pollutants can create serious effects. And, experimentation can range even farther afield from the study of fishes. As just one example, despite the increasing ease of experimentation on larval fishes (see Section 4.3 above), the activities of the RECOVER Consortium developmental biologists encompass the very deeply understood avian embryo model. From the days of Aristotle observers have investigated morphological and physiological aspects of development in the chicken egg, for example, and as a consequence, the normal and abnormal physiological development of the avian embryo is deeply understood (for review see [122]). By using well-honed techniques such as *in vitro* or so-called "shell-less" cultures of chicken embryos to study the first few days of cardiovascular development ([123]), we are able to measure cardiovascular system changes that can be placed in the context of a very well

understood system.

5. Conclusions: The Advantages of Interdisciplinary Cooperation in Fisheries, Aquaculture and Pollution Research

Biology, from its emergence as a formal discipline, has always lent itself to interdisciplinary activities involving chemistry, mathematics, engineering, etc. [124]. Collaboration between scientists is nothing new. Not sur- prisingly, then, modern fisheries and aquaculture as subsets of the life sciences have benefited from strategic interactions with researchers in different disciplines within the basic sciences. Certainly, an understanding of the developmental biology of wild-harvested and especially cultured species is crucial to any economically viable operation. Additionally, understanding the influence of both natural and anthropogenic environmental factors on animal populations is critical to commercial operations as well as for maintaining wild healthy populations. Finally, physiology is a key discipline that integrates phenotypic changes at the molecular, morphological, and behavioral levels to allow animals to survive in a challenging environment.

While most would acknowledge the importance of the disciplines mentioned above (and many others, such as toxicology, chemistry, etc.), collectively we have not developed a comprehensive understanding of the interactions between these various disciplines. There are certainly synergies to be identified and pursued among these areas in terms of understanding how organisms live in a complex, variable environment and how they respond to complex chemical assault from pollutants, such as the crude oil released by the Deepwater Horizon Oil Spill. To this end, the RECOVER Consortium is specifically designed to create highly coordinated, interdisciplinary teams to address key questions in economically important fish species that would not otherwise be feasible research goals. Additional synergies will also come from RECOVER's developmental biologists, environmentalists and physiologists working together with physical scientists and others in organized research teams that integrate scientific disciplines to further the understanding of the complex interactions that alter the ecosystem at large.

It is in this spirit of cooperation and collaboration between RECOVER life scientist teams, as opposed to individual and potentially isolated efforts, that we part with a final thought evoked in this old saying "The whole is greater than the sum of the parts".

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