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# ET&C Perspectives

THE PERSPECTIVES COLUMN IS A REGULAR SERIES DESIGNED TO DISCUSS AND EVALUATE POTENTIALLY COMPETING VIEWPOINTS AND RESEARCH FINDINGS ON CURRENT ENVIRONMENTAL ISSUES

*The Challenge:* "Bridging the gap" with fish: Advances in assessing exposure and effects across biological scales

There is now a broad consensus that many contaminants—including certain pesticides, pharmaceuticals, and industrial compounds—have deleterious effects in fish. Biological responses to toxicants can range from the subcellular to the population scales and extend beyond single species to multiple trophic levels. The goal of many current research initiatives is to bridge the gap between molecular initiating events (such as receptor binding) and ensuing adverse outcomes at higher and more complex levels of biological organization, particularly those that determine individual survival and reproduction, population growth and abundance, and aquatic community structure and function. In the present column, we highlight the perspectives of several established researchers in the field of ecotoxicology, each targeting a different aspect of this multifaceted problem influencing aquatic ecosystems.

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*In Response*: Scaling polycyclic aromatic hydrocarbon toxicity to fish early life stages: A governmental perspective

The 1989 *Exxon Valdez* disaster oiled the shoreline spawning habitats for pink salmon and Pacific herring in Prince William Sound, Alaska (USA). Research on these 2 species in subsequent years showed that crude oil–derived polycyclic aromatic hydrocarbons (PAHs) are highly toxic to developing embryos and larvae (e.g., Carls et al. [1] and Marty et al. [2]). The fundamental aspects of crude oil toxicity syndrome are now well established at the scale of the individual animal for a wide diversity of fish species exposed to crude oils from different geological origins worldwide. Across species, the syndrome is consistently characterized by defects in heart formation and function as well as craniofacial and finfold abnormalities (Figure 1).

In more recent years, the zebrafish model has provided a versatile experimental system for identifying which PAHs in complex mixtures are causal. These single-compound studies have shown that the canonical toxicity syndrome can be attributed primarily to PAHs having 3 rings, including the phenanthrenes, dibenzothiophenes, and fluorenes. Using gene-silencing methods, it was also shown that the developing heart is the primary target organ for tricyclic PAH toxicity [3]. For single compounds and whole crude oil alike, the cardiotoxicity in embryos and larvae varies in severity across a gradient of exposure concentrations. The injury phenotype ranges from a slowed heartbeat (bradycardia) to arrhythmias, contractility defects, atrium-to-ventricle conduction blockade, and eventually heart failure as evidenced by pericardial fluid accumulation (edema; arrows in Figure 1) [3–5]. The cardiac phenotype is more severe in gene knockdown experiments wherein the expression of PAH-metabolizing cytochrome p450 enzymes (e.g., CYP1A) is suppressed, providing further evidence that crude oil cardiotoxicity is mediated by PAHs [6].

Tricyclic PAH toxicity to the developing fish heart has now been documented in freshwater and marine species, from subtropical to sub-Arctic habitats. As crude oil weathers, the tricyclic fraction is enriched, yielding a proportional increase in cardiotoxic potential on a mass-equivalent basis. The characteristics of the syndrome are very similar across species, irrespective of whether fish are exposed to crude oil in the field, effluent from oiled gravel columns, or oil and water mixed mechanically [4,5,7–9]. Diffusion of PAHs through oil droplet exclusion matrices is sufficient to cause developmental defects, and thus physical contact with oil is not necessary [10]. Moreover, tricyclic PAHs from other sources, including maritime vessel spills (residual fuel oils), creosote, and urban

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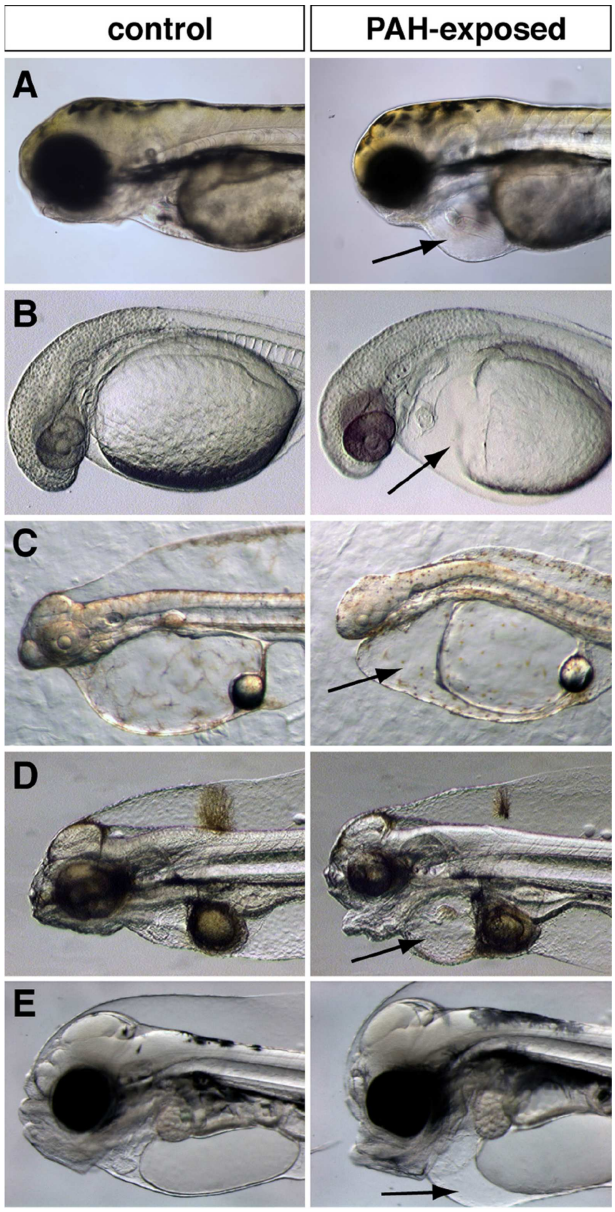


Figure 1. Polycyclic aromatic hydrocarbons (PAHs) derived from crude oils from different geological sources worldwide cause a consistent heart failure injury phenotype across a diversity of fish species. All images are hatching-stage larvae (**A**, **C–E**) or late embryos (**B**) exposed to crude oil starting shortly after fertilization, with representative controls in the left column and oil-exposed fish in the right column. Arrows indicate pericardial fluid accumulation or edema as an indication of circulatory failure. (**A**) zebrafish (*Danio rerio*) and Louisiana (USA) sweet crude oil; (**B**) Pacific herring (*Clupea pallasi*) and Alaska North Slope (USA) crude oil, (**C**) olive flounder (*Paralichthys olivaceous*) and Iranian heavy crude oil, (**D**) yellowfin tuna (*Thunnus albacares*) and Louisiana (USA) sweet crude oil, and (**E**) Atlantic haddock (*Melanogrammus aeglefinus*) and Norwegian Sea crude oil. PAH = polycyclic aromatic hydrocarbon.

stormwater runoff, cause the familiar cardiac dysregulation in developing fish.

The specific features of the developmental toxicity syndrome at the scale of the whole organism, together with the nature of the functional defects at the scale of the target organ (the heart), suggested that tricyclic PAHs might act pharmacologically on discrete ion channels or related ion transport machinery in heart muscle cells. For example, the injury phenotype closely resembles the zebrafish *breakdance* loss-of-function mutant lacking the delayed rectifying potassium current that is essential for cardiomyocyte repolarization [3]. Moreover, the progression from bradycardia to arrhythmia suggests a disruption of the normal cycling of heart muscle cell depolarization, contraction, and repolarization that underpins a normal heart rhythm. This was recently confirmed at the cellular scale using isolated cardiomyocytes from juvenile bluefin and yellowfin tunas. Crude oil samples collected from the Deepwater Horizon oil spill were shown to disrupt excitation–contraction coupling via the blockade of internal calcium cycling and the repolarizing potassium conductance [11].

Fish embryos with deformed jaws and heart failure will not survive to become free-swimming and feeding larvae. For comparatively lower levels of PAHs, however, embryos will survive transient exposures with only mild effects on heart rhythm or contractility. These effects, while proximally sublethal, can nevertheless permanently reduce cardiorespiratory performance at later life stages. During the developmental phase of heart morphogenesis, form depends inextricably on function. Sublethal PAH effects on circulatory function during this period of morphogenesis can lead to permanent changes in heart shape [6]. This delayed form of toxicity likely explains the increased mortality of juvenile Alaska pink salmon exposed transiently to crude oil as embryos and then released into the ocean in large-scale mark and recapture studies. Although outwardly normal at the time of release (smolt stage), the oil-exposed treatment groups showed a consistent and significant reduction in marine survival [12].

The current state of the science is now focused on linkages across biological scales, from channel blockade and the disruption of excitation–contraction coupling in individual heart muscle cells to heart development, circulatory and respiratory performance, delayed mortality of individual fish, and associated consequences for the abundance and dynamics of wild populations. For forage fish such as Pacific herring, the biological scaling will eventually extend to communities and ecosystems. Using herring as an example, the path ahead will combine empirical data on PAH cardiotoxicity with individual-based modeling, including the use of bioenergetics models to couple cardiac output, respiratory function, growth, and first-year survival. Individual survival estimates can then provide a foundation for population-scale modeling as well as ecosystem-based models to assess the influence of forage fish abundance on populations of birds, marine mammals, fish, and other predators within marine communities. Empirically based models are likely to improve the natural resource injury assessment capability for future oil spills and may shed light on the collapse of fisheries in the aftermath of past spills. This includes the precipitous decline of the Prince William Sound herring fishery 3 yr to 4 yr after the *Exxon Valdez* disaster [13], at a time when herring spawned in the vicinity of oiled shoreline habitats would have recruited into the adult population.

Lastly, advances in molecular biology and postgenomic technologies will continue to improve the resolving power of future PAH injury studies in fish across multiple scales. This includes the identification of suites of genes with expression patterns that are phenotypically anchored to the alterations in cardiovascular function and anatomy that have been documented as either immediate or delayed consequences of embryonic PAH exposure. At the molecular level, this will likely yield a more detailed understanding of how a transient pharmacological blockade of excitation–contraction coupling in cardiomyocytes ultimately changes the trajectory of heart morphogenesis. New transcriptional indicators of cardiovascular stress may ultimately prove more sensitive than conventional

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morphological and functional measures and more amenable to high-throughput screening. At the organismal scale, the development of a new generation of molecular biomarkers diagnostic for tricyclic PAH exposure and cardiotoxic injury in wild fish remains a long-standing goal. These will enhance future environmental surveillance efforts in fish spawning habitats that are vulnerable to PAH inputs from oil spills, land-based runoff, and other sources. This includes, for example, circumpolar species likely to be affected by accidents involving the maritime shipping and resource extraction activities that are becoming increasingly more intensive in the Arctic.

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## *In Response:* Environmental and biological considerations for active pharmaceutical ingredients in the environment and their effects across multiple biological scales: An academic perspective

As a group of chemicals of emerging concern in the aquatic environment, human and veterinary pharmaceuticals are as diverse as their uses and include the active pharmaceutical ingredients in analgesics, antibiotics, antiepileptics, antihypertensives, lipid regulators, antidepressants, antianxiety medications, hormone therapies, and many others [1]. These chemicals are either discharged with domestic and hospital wastewaters or part of overland runoff in areas where intense livestock production occurs. Broad-scale surveys in the United States have found active pharmaceutical ingredients in up to 40% of surface water samples [2], and the types detected continue to increase as our ability to measure them improves. Environmental concentrations generally range from subnanograms per liter to low micrograms per liter in downstream waters, with considerable variability in the mixtures and concentrations from one location to another [1–3]. Their presence has raised concern because of the evidence that some persist in waters and sediments and bioaccumulate in aquatic species (e.g., the antiepileptic carbamazepine); interfere with sexual development, reproduction, and behaviors in fish (e.g., an active ingredient in the birth control pill, 17α-ethinyl estradiol [EE2]); and affect primary producers (e.g., the antibiotic tetracycline). However, much of what we know about their effects is based on lab studies on a limited number of compounds for a small subset of species [4].

The waters become much more muddied when we consider how environmental and biological factors may modify either the uptake or toxicity of these active pharmaceutical ingredients to aquatic life as well as the potentially additive effects of the mixtures present in municipal wastewaters or agricultural runoff. As our knowledge of the presence, fate, and effects of active pharmaceutical ingredients advances, so too does the evidence that the metabolism of these chemicals in the environment, the dose to which organisms are exposed, and the health outcomes of drugs on species in surface waters requires consideration of the characteristics of the systems and species themselves. The following examines some of the environmental and biological factors that warrant consideration because they may reduce or increase the impacts of active pharmaceutical ingredients in aquatic environments and put organisms from some systems at greater risk than others. Although many of these factors are reasonably well understood for other contaminants (but see Clements et al. [5]), as described below, environmental transformations and organism metabolism and toxicity of active pharmaceutical ingredients can be unexpected.

Human and veterinary pharmaceuticals are not immune to many of the physical processes known to affect the fate of other chemicals in the environment. Direct and indirect photolysis and biotransformation occur, but the rate of active pharmaceutical ingredient removal will vary with the inherent properties of

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