Behaviour Revised: Contaminant Effects on Aquatic Animal Behaviour

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The field of aquatic ecotoxicology has matured over the past several decades. It has gone from simple "pickle jar" science in the 1960s and '70s, to a much more sophisticated and mechanistic discipline with the advent of molecular and genomic tools. A long-standing challenge for aquatic ecotoxicology has always been to extrapolate results generated under controlled laboratory conditions to the variable conditions of the field. Attempts at making lab-to-field extrapolations have had mixed success. Laboratory studies conducted at the molecular level have had difficulties translating to natural conditions, whereas field studies often lack a mechanistic understanding of potential toxicity.

Behavioural toxicology has emerged as a promising discipline to bridge the laboratory-to-field divide. Animal behaviour integrates the internal physiology of the animal and the external conditions of the environment. Toxicant-induced behavioural impairments often point to underlying physiological deficits that can be used effectively to evaluate ecological risk—especially if the affected behaviour relates directly to survival, growth, or reproduction.

Environmental regulators and industry have been reluctant to adopt behavioural endpoints for risk assessment purposes, mainly because of a current lack of standardized

approaches or perceived difficulties extrapolating lab-based results to natural receiving environments. However, the ecological relevance of behavioural endpoints is clear given that they relate directly to survival, growth, and reproduction. In an attempt to advance the field of behavioural toxicology, particularly as it relates to environmental compliance and risk assessment, we held a special symposium entitled, "Behaviour Revised: Contaminant Effects on Aquatic Animal Behaviour," at the 25th annual meeting of the Society of Environmental Toxicology and Chemistry (SETAC) Europe meeting, held in Barcelona, Spain (May 3 - 7, 2015). We invited a range of behavioural toxicologists from around the world to contribute towards advancing our understanding of how environmental toxicants affect animal behaviours with a mind towards advancing the discipline for the purposes of environmental regulation and ecological risk assessment. It was at this meeting that we began soliciting manuscripts for this special issue of *Aquatic Toxicology*, both from the participants at the special symposium, as well as from other experts from around the world. The collection of papers in this special issue is from those that answered the call.

Increased pharmaceutical use in humans and domestic animals has led to a concomitant increase in the presence of the same pharmaceuticals and their metabolites in aquatic systems. Pharmaceuticals are now considered to be 'emerging' contaminants of concern in aquatic ecosystems. Henriques et al. (2016) report that locomotor function is impaired in zebrafish embryos exposed to gemfibrozil—a pharmaceutical lipid regulator—at concentrations that are significantly lower than those required to induce developmental anomalies. Similarly low concentrations of carbamazepine, diazepam, propranolol, and fluoxetine, in the ng/L range, were sufficient to impair the phototactic

response of *Daphnia magna* (Rivetti et al., 2016). Androgenic growth hormones, such as 17β-trenbolone are frequently administered to cattle as growth promoters. However, as Tomkins et al. (2016) demonstrate, concentrations as low as 4 ng/L are sufficient to affect female mate choice in wild guppies. These pharmaceutical-induced effects on locomotion and phototaxis could result in an impaired ability to respond to predators. Effects on female mate choice could affect reproductive output as well as the genetic integrity of offspring.

Metals are also well known neurotoxicants that can affect animal behaviour. Pereira et al. (2016) linked changes in white sea bream brain morphology following exposure to inorganic mercury to impaired motor function and anxiety status. Although some brain regions could recover once the mercury was removed, others could not, resulting in some long-term behavioural deficits. Ašmonaitė et al. (2016) present a novel approach using early life-stage zebrafish locomotion from video-tracking software and multivariate statistical analyses to develop heat maps for fish exposed to both silver ions and nanoparticles. Their approach allowed them to differentiate ion effects from nanoparticles, and different behavioural profiles associated with exposure concentrations; hypoactivity at high concentrations, and hyperactivity at low concentrations. Gauthier et al. (2016) followed-up a previous study (Gauthier et al., 2015) that showed that mixtures of copper and phenanthrene (a polycyclic aromatic hydrocarbon; PAH) causes potentiated toxicity in the freshwater scud, *Hyalella azteca*. Here, they demonstrate that copper and phenanthrene act independently to induce toxicity, and the behavioural deficits induced by phenanthrene exposure (e.g., hyperactive, uncoordinated activity) result from an acetylcholinesterase inhibition, much like an organophosphate pesticide.

Pesticides can also lead to several behavioural toxicological effects. Andrade et al. (2016) exposed early life-stage zebrafish to carbendazim, a broad-spectrum fungicide, and demonstrated that behavioural impairments occurred at exposure concentrations that were orders of magnitude lower than those required to affect development, metabolism, or lethality. Rodrigues et al. (2016) exposed freshwater planaria to chlorantraniliprole—an anthranilic diamide insecticide—and demonstrated impaired feeding and swimming behaviours using a video tracking system at low, ecologically relevant exposure concentrations. Renick et al. (2016) tested the interactive effects of an organophosphate pesticide, chlorpyrifos, and a common trematode parasite, *Euhaplorchis californiensis*, in Californian coastal waters on the California killifish. The pesticide reduced the activity of the neurotransmitter acetylcholinesterase, resulting in reduced swimming speeds; whereas the trematode had no effect on swimming behaviour.

Contaminants associated with municipal and industrial waste waters can also cause behavioural effects in aquatic organisms. Lanctôt et al. (2016) demonstrated that striped marsh frog tadpoles exposed to coal mine waste water for a short duration (24 h) became hyperactive. However, this effect was transient and was not detectable after 28 days of exposure. Lari et al. (2016) showed that Caspian roach exposed to the water soluble fraction of crude oil show impaired swimming performance, which could be linked to contaminant-induced impairments in the oxygen delivery system (e.g., decreased pO_2 and increased pCO_2 in oil exposed animals). Melvin (2016) showed increased swimming velocity and body condition, and decreased lipid content in juvenile empire gudgeons exposed to full-strength municipal waste water.

The recent emergence of behavioural toxicology as a viable approach to integrate lab-

97 and-field has given rise to a number of different tools that will be useful as we begin to
98 develop into the area of regulatory compliance. Hartmann et al. (2016) present a novel
99 method for using freshwater mussel behaviour, such as filtration, valve transition
100 frequency (from open to closed), and contaminant avoidance, as viable ecotoxicological
101 endpoints for chemical exposures, temperature changes, and particulate matter
102 contamination. Kristofco et al. (2016) address the lack of high-throughput screening
103 assays available for behavioural toxicology, and demonstrate that understanding specific
104 life-stage responses can lead to more sensitive behavioural toxicological endpoints. By
105 testing several zebrafish developmental stages, they demonstrate that larvae 10 days post
106 hatch (dph) are significantly more sensitive to diazinon (pesticide) and diphenhydramine
107 (pharmaceutical) than earlier, embryonic life-stages known to be more sensitive in
108 growth and survival tests. Kaluef et al. (2016) argue that zebrafish neurobehavioural
109 phenomics is a promising new approach that integrates behavioural phenotypes with
110 various genetic and environmental factors. They specifically advocate for zebrafish as a
111 model owing to its homology with the human genome and the wide range of genomic and
112 molecular tools available for immediate experimentation. Michalec et al. (2016) describe
113 the use of lipid nanocapsules to deliver hydrophobic toxicants (PCBs and PAHs) to
114 calanoid copepods. The new delivery system allows for an analysis of the relative
115 contribution of water and dietary toxicant exposure routes as they might affect 3-D
116 copepod swimming behaviour.
117 Several authors in this issue provide critical reviews related to different aspects of
118 behavioural toxicology. Parker (2016) argues against methodological standardization for
119 behavioural toxicology assays given the numerous confounding variables that can

influence animal behaviour. Instead, he offers approaches that make use of the test animal's unconditioned natural behavioural tendencies, which can increase the reliability and ecological validity of behavioural analyses. Hellström et al. (2016) provide a review of current acoustic telemetry tools available to monitor animal behaviour in natural systems. These tools provide a direct link between results generated under tightly controlled laboratory conditions to the realities of natural systems. Tierney (2016) reviews the literature on fish behavioural avoidance responses to contaminants in the environment. He demonstrates that avoidance responses are common, but not universal. Several studies have documented cases where fishes are attracted to contaminants at concentrations that are likely maladaptive. He then provides a framework on which to base future studies to screen fish species for their ability to detect and avoid environmental contaminants.

Together, these papers comprise a snapshot of the current state of aquatic behavioural toxicology. Advances in our ability to track animal behaviour using sophisticated video and high-throughput tracking systems has increased the resolution with which contaminant-induced behavioural deficits can be measured. New animal models and novel contaminant-delivery systems provide researchers with a well-supplied tool chest for improving the ecological relevance of environmental risk assessments. Genomic and molecular tools provide a mechanistic underpinning of behavioural effects resulting from contaminant exposures. Behavioural toxicology is no longer an emerging field. We now understand that environmental contaminants—including pharmaceuticals, metals, pesticides, and municipal and industrial waste waters, among others—can affect aquatic animal behaviour resulting in maladaptive effects that need to be considered by

environmental managers and government regulators. Our next challenges include linking behavioural effects observed under controlled laboratory conditions to natural systems, and to adopt well-established contaminant-induced behavioural deficits into regulatory compliance legislation.

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