Frontiers in quantifying wildlife behavioural responses to chemical pollution

Michael G. Bertram1, Jake M. Martin2, Erin S. McCallum1, Lesley A. Alton2, Jack A. Brand2, Bryan W. Brooks3, Daniel Cerveny1,4, Jerker Fick5, Alex T. Ford6, Gustav Hellström1, Marcus Michelangeli1,7, Shinichi Nakagawa8, Giovanni Polverino2,9,10, Minna Saaristo11, Andrew Sih7, Hung Tan2, Charles R. Tyler12, Bob B.M. Wong2, and Tomas Brodin1

1 Department of Wildlife, Fish, and Environmental Studies, Swedish University of Agricultural Sciences, Skogmarksgränd 17, Umeå, Västerbotten, SE-907 36, Sweden
2 School of Biological Sciences, Monash University, 25 Rainforest Walk, Melbourne, Victoria, 3800, Australia
3 Department of Environmental Science, Baylor University, One Bear Place, Waco, Texas, 76798-7266, U.S.A.
4 Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, University of South Bohemia in Ceske Budejovice, Zátiší 728/II, Vodnany, 389 25, Czech Republic
5 Department of Chemistry, Umeå University, Linnaeus väg 10, Umeå, Västerbotten, SE-907 36, Sweden
6 Institute of Marine Sciences, University of Portsmouth, Winston Churchill Avenue, Portsmouth, Hampshire, PO1 2UP, U.K.
7 Department of Environmental Science and Policy, University of California, 350 E Quad, Davis, California, CA, 95616, U.S.A.
8 Evolution & Ecology Research Centre, School of Biological, Earth and Environmental Sciences, University of New South Wales, Biological Sciences West (D26), Sydney, NSW, 2052, Australia
9 Centre for Evolutionary Biology, School of Biological Sciences, University of Western Australia, 33 Stirling Highway, Perth, WA, 6009, Australia
10 Department of Ecological and Biological Sciences, Tuscia University, Via S.M. in Gradi n. A, Viterbo, Lazio, 01100, Italy
11 Environment Protection Authority Victoria, EPA Science, 2 Terrace Way, Macleod, Victoria, 3085, Australia
12 Biosciences, College of Life and Environmental Sciences, University of Exeter, Stocker Road, Exeter, Devon, EX4 4QD, U.K.

ABSTRACT

Animal behaviour is remarkably sensitive to disruption by chemical pollution, with widespread implications for ecological and evolutionary processes in contaminated wildlife populations. However, conventional approaches applied to study the impacts of chemical pollutants on wildlife behaviour seldom address the complexity of natural environments in which contamination occurs. The aim of this review is to guide the rapidly developing field of behavioural ecotoxicology towards increased environmental realism, ecological complexity, and mechanistic understanding. We identify research areas in ecology that to date have been largely overlooked within behavioural ecotoxicology but which promise to yield valuable insights, including within- and among-individual variation, social networks and collective behaviour, and multi-stressor interactions. Further, we feature methodological and technological innovations that enable the collection of data on pollutant-induced behavioural changes at an unprecedented resolution and scale in the laboratory and the field. In an era of rapid environmental change, there is an urgent need to advance our understanding of the real-world impacts of chemical pollution on wildlife behaviour. This review therefore provides a roadmap of the major outstanding questions in behavioural ecotoxicology and highlights the need for increased cross-talk with other disciplines in order to find the answers.

Key words: animal, behaviour, contaminant, ecology, ecotoxicology, environmental change, fitness, pollutant, population, wildlife

* Address for correspondence (Tel: +46 (0)70 446 90 55; E-mail: michael.bertram@slu.se)
CONTENTS

I Introduction ................................................................. 1347
II Increasing environmental realism ........................................... 1348
(1) Confronting real-world exposure scenarios ................................. 1348
(2) Prioritising contaminants of concern ........................................... 1348
III Addressing ecological complexity ............................................ 1349
(1) Key experimental design considerations ...................................... 1349
(2) Novel research directions in behavioural ecotoxicology: what are the knowns and the unknowns? 1349
IV Tools and technologies for advancing behavioural ecotoxicology ............................................ 1355
(1) Laboratory-based experimental innovations ................................ 1355
(2) Scaling up: experimental advances in the field ................................ 1356
V Improving statistical sophistication ........................................... 1357
(1) Embracing mixed modelling to address biological complexity .......... 1357
(2) Harnessing meta-analytic approaches ........................................ 1358
VI Conclusions .................................................................. 1358
VII Acknowledgements .......................................................... 1358
VIII References ................................................................. 1358
IX Supporting information ..................................................... 1364

I INTRODUCTION

Chemical pollution represents a leading threat to human health, wildlife, and ecosystems around the world (Landrigan et al., 2018). In fact, increases in the diversity and volume of novel synthetic chemicals released into the environment now far outpace other key drivers of global change (e.g. rising atmospheric CO₂, habitat loss; Bernhardt, Rosi & Gessner, 2017). This trend is the result of rapidly increasing chemical use, with the global chemicals industry, currently valued at >US$5 trillion, projected to double in size between 2017 and 2030 (UNEP, 2019). The spread of novel environmental contaminants is concerning given that many ecosystems are already inundated with myriad organic and inorganic chemicals released by human activities, including metals, pesticides, pharmaceuticals, and per- and polyfluoroalkyl substances (PFAS) (Bertram et al., 2022). Moreover, chemical pollution continues to accelerate large-scale wildlife losses (Groh et al., 2022), including dramatic declines in the abundance and biodiversity of insects (Sánchez-Bayo & Wyckhuys, 2019), avifauna (Rosenberg et al., 2019), and aquatic species (Tickner et al., 2020).

Adverse impacts of chemical pollution on wildlife are not limited to mortality at acute toxic levels. Indeed, exposure to environmental pollutants at sublethal concentrations acts as an often-cryptic driver of wildlife declines by disrupting a wide range of fundamental biological processes [e.g. development (Gore et al., 2015); reproduction (Aulsebrook et al., 2020)]. In this regard, a growing body of research has demonstrated that chemical pollutants can have both direct and indirect impacts at multiple levels of organisation by altering animal behaviour (reviewed in Saaristo et al., 2018). In fact, behaviour can be remarkably sensitive to perturbation by even low pollutant concentrations, and is often disturbed at much lower exposure levels than more conventional endpoints in ecotoxicology, such as development, reproduction, and mortality, which is routinely estimated using LC₅₀ (i.e. the concentration at which 50% of the animals in an exposed population are expected to die) (Melvin & Wilson, 2013). This is alarming given that behaviour represents the link between an organism and its environment, meaning that an inability to appropriately produce and maintain behaviour can have dire consequences for individual- and population-level fitness (Wong & Candolin, 2015).

Clearly, there is an urgent need to better establish the causes and consequences of pollutant-induced behavioural changes in wildlife (discussed in Peterson et al., 2017). However, conventional approaches applied in behavioural ecotoxicology are often insufficient to address the complexity of real-world exposure scenarios (Pyle & Ford, 2017), that is exposing individuals of a single species to a single contaminant and measuring movement in a confined arena. This limits our ability to achieve meaningful biological interpretations of pollutant impacts at the individual level. Moreover, such approaches hamper the accurate extrapolation of findings to predict effects on populations and communities, which is vital to environmental protection efforts (Ford et al., 2021).

At the same time, significant methodological and technological innovations promise to transform the study of wildlife behavioural responses to pollutants, originating from within behavioural ecotoxicology and related disciplines such as analytical, environmental, and computational chemistry, comparative toxicology, behavioural ecology, collective behaviour, movement ecology, automated sensing, and computer vision. These advances open the door to the collection of data of high environmental and ecological relevance at an unprecedented resolution and scale in both the laboratory and the field. Crucially, this is enabling behavioural ecotoxicologists to confront the formidable complexity that often characterises real-world exposure scenarios (e.g. chemical interactions, chemical and non-chemical stressor interactions, complex and/or large-scale behaviours). Indeed, recent work
embracing these innovations has revealed effects of pollutants on behavioural processes that were previously not possible to capture using conventional approaches. These range from neurotoxic insecticide-induced migration delays in songbirds (Eng, Stutchbury & Morrissey, 2019) to the breakdown of behavioural diversity in fish populations exposed to pharmaceutical pollution (Polverino et al., 2021).

The aim of this review is to guide the field of behavioural ecotoxicology towards increased environmental realism, ecological complexity, and mechanistic understanding by highlighting major research opportunities and experimental innovations. First, we examine how recent advances in analytical and environmental chemistry can increase environmental realism and experimental quality in behavioural ecotoxicology, and discuss underused approaches to prioritising contaminants of concern for behavioural testing. We then argue for targeting behavioural endpoints of high ecological significance, and outline concepts in ecology that have been broadly overlooked in behavioural ecotoxicology to date but which have the potential to provide key insights. We go on to present a suite of cutting-edge tools and technologies that can be incorporated into behavioural ecotoxicology studies during design, implementation, and analysis. Further, we consider developments in statistical sophistication necessary to evaluate behavioural impacts of pollutants occurring in complex systems. Finally, we provide open-access and freely available tools and resources, wherever possible, in order to increase the accessibility of behavioural ecotoxicology research.

II INCREASING ENVIRONMENTAL REALISM

Here, we outline how recent advances in analytical and environmental chemistry can be harnessed to enhance environmental realism and experimental quality in behavioural ecotoxicology, and discuss underused approaches for prioritising contaminants and mixtures of concern for behavioural testing.

(1) Confronting real-world exposure scenarios

Given the sheer number of chemicals in commerce, one of the challenges we face is how to identify what potentially behaviour-modifying chemicals and mixtures are present in the environment and at what levels. There are at least 350,000 chemicals and chemical combinations registered for use around the world (Wang et al., 2020), several thousand of which have been detected in the environment (Hollender et al., 2017). However, regulatory monitoring is still limited to a small selection of well-known contaminants, representing only a fraction of overall chemical and mixture risk (discussed in Brack et al., 2019a). For example, the United States Clean Water Act regulates 126 priority pollutants (EPA, 2021a), while the European Water Framework Directive is based on the analysis of 43 priority substances, as well as pollutants (~300 total) defined nationally by the different European Union member states (European Union, 2013). This limitation is partly a consequence of traditional environmental surveillance methodologies relying on targeted analyses of contaminants (discussed in Brack et al., 2019b), which requires that compounds are already ‘known’ and that chemical analytical standards are available to support their measurement. Fortunately, rapid advances in analytical and environmental chemistry, such as non-targeted screening using high-resolution mass spectrometry coupled to chromatography, now allow for near-simultaneous identification of thousands of contaminants that are not preselected for study across a range of environmental compartments (e.g. water, air, dust, soil) and biological matrices (e.g. tissue, blood, plasma) (Hollender et al., 2017; McCord, Groff II & Sobus, 2022). The sensitivity of analytical instrumentation for both targeted and non-targeted analyses has also increased markedly, allowing for the detection of even trace concentrations (e.g. pg/l or kg, ng/l or kg) of chemicals that could cause sub-lethal changes to animal behaviour (Jiang & Li, 2020). However, equipment and analysis costs are still a limiting factor in the widespread application of these approaches (see Fernandez, Andre & Cardeal, 2020). Thankfully, data on complex exposure scenarios in the wild are also increasingly accessible due to the availability of large-scale, open-access databases documenting the occurrence of environmental contaminants, for example the EPA NCOD Database (EPA, 2021b), the NORMAN EMPODAT Database (NORMAN, 2022), the UBA Pharmaceuticals in the Environment Database (UBA, 2021), and the Global Monitoring of Pharmaceuticals Project (Wilkinson et al., 2022). Taken together, these developments increasingly allow behavioural ecotoxicologists to place their work in a real-world context when targeting contaminants and mixtures of concern (e.g. based on environmental prevalence, persistence, co-occurrence, and/or relevance to particular species or research questions). This targeted approach is especially important given that testing all relevant chemicals and combinations of chemicals for potential behavioural effects would be prohibitively costly, inefficient, and ethically problematic. Moreover, these advances facilitate extreme precision, accuracy, and breadth in characterising and validating exposure scenarios (e.g. confirming contaminants and transformation products in exposure media and/or experimental animal tissues).

(2) Prioritising contaminants of concern

The next major challenge is identifying and prioritising contaminants and mixtures that are likely to have deleterious effects on wildlife behaviour. To this end, we must look beyond traditional laboratory exposure experiments alone, again adopting a targeted approach given that more chemicals exist than can be adequately examined in a timely manner. It is therefore crucial to predict how chemicals interact with biological systems, which requires developing predictive approaches to identify and prioritise contaminants and
mixtures that are likely (or not) to alter behaviour. In addition to facilitating the analytical chemistry advances discussed previously, computational chemistry can help to identify inherent chemical attributes and properties that are likely to interact with specific biomolecules and elicit downstream biological and behavioural responses in organisms. For example, an explosion of molecular genetic information across phyla is facilitating in silico advances in predictive toxicology and pharmacology during drug development (Raias & Bajic, 2016). Such approaches are translatable from human to environmental protection efforts when the structural alerts for specific chemicals align with predictions of evolutionarily conserved molecular initiating events (MIEs, i.e. a molecular interaction between a chemical and a specific biomolecule; Lalone et al., 2016). These MIEs may then lead to a sequential series of higher-order effects (e.g. gene activation, changed cell signalling, disturbed homeostasis, altered tissue development and/or function) across biological scales (e.g. molecular, cellular, tissue, and/or organ levels). This may produce adverse outcomes – for instance, on behaviour – of relevance to individuals and populations [summarised in the ‘Adverse Outcome Pathway’ (AOP) concept; Ankley et al., 2010]. Adding a layer of complexity, AOPs can also be used to predict mixture effects (reviewed in Escher et al., 2017), which may be produced by combinations of chemicals that act via the same or different MIEs, and could include additive, antagonistic, or synergistic interactive effects on animal behaviour. Supporting this approach, large-scale computational toxicology initiatives are examining thousands of chemicals with hundreds of targets in in vitro models to identify MIEs that may cause adverse outcomes in the environment (e.g. Tox21, 2020; ToxCast, 2020; ToxPi, 2021). Although very little integration of computational chemistry, comparative toxicology, and behavioural ecotoxicology has occurred to date, this approach could be extremely valuable in facilitating effective prioritisation of contaminants of concern for behavioural testing, and promises to improve mechanistic understanding. Moreover, because groups of chemicals within contaminant classes often act on the same or similar physiological pathways and molecular mechanisms (e.g. serotonin re-uptake inhibiting pharmaceuticals; Gunnarsson et al., 2019), this targeted approach promises to reduce substantially the number of candidate chemicals and mixtures for behavioural testing.

III ADDRESSING ECOLOGICAL COMPLEXITY

A common critique of behavioural ecotoxicology research is the perception that such studies lack sufficient ecological relevance to be applied to real-world scenarios (discussed in Ågerstrand et al., 2020). This is true even though, when assessed correctly, behavioural responses can be invaluable in predicting higher-order population- and community-level outcomes (Saaristo et al., 2018). Here, we outline practices to improve ecological realism in behavioural ecotoxicology research and highlight seldom-studied but significant new research avenues in this field.

(1) Key experimental design considerations

When designing studies testing the potential behavioural impacts of contaminants, how do we decide which behaviours we should test and how to test them most appropriately? Most importantly, behaviours need to be ecologically relevant to the focal species and must be biologically targeted by the contaminant(s) in question via some direct or indirect mechanism(s) of action, and/or predicted to impact an organism’s spatial pollution attraction/avoidance (discussed in Araújo et al., 2020). Contaminants that are expected to affect fundamental behaviours for a species’ ecology and fitness should be prioritised, as these will likely have more immediate and adverse impacts. For instance, contaminants that target social-cue mechanisms may disproportionately affect organisms that rely on social information and/or the ability to form cohesive groups (e.g. schools, flocks) that are essential to their antipredator behaviour, feeding and foraging, and mating biology (e.g. Ward et al., 2008; Martin et al., 2019; Mason et al., 2021). Similarly, certain pollutants might interfere with a species’ ability to avoid predators by targeting neural pathways involved in predator recognition (e.g. Polo-Cavia, Burraco & Gomez-Mestre, 2016) or that support locomotion for escape (e.g. Sievers et al., 2018). Further, behavioural experiments should ideally be tailored to mimic the species’ natural social environment and habitat (or be conducted in the wild; see Section IV.2). Social species, for instance, should be tested in the presence of conspecifics (see Martin & McCallum, 2021), both to increase ecological relevance and because of the known impacts of social isolation on animal physiology and behaviour (e.g. Shams, Chatterjee & Gerlai, 2015; Tunbak et al., 2020; Munson, Michelangeli & Sih, 2021). Likewise, species that rely on specific environmental resources as part of their behavioural repertoire should be tested with those resources (e.g. refuge structures, substrate, perches), and if movement-related behaviours are being measured then adequately sized experimental environments should be provided for natural space use and avoidance behaviours to occur. These details will vary from species to species but their absence may lead to unnatural behaviours and erroneous biological interpretations.

(2) Novel research directions in behavioural ecotoxicology: what are the knowns and the unknowns?

Keeping the above experimental considerations in mind, we next feature a range of future research avenues that, despite being of high ecological significance, have received relatively little attention in behavioural ecotoxicology (Fig. 1; see also Table 1 for examples of studies that have been carried out in each of these research areas to date). We by no means see this as an exhaustive list or as mutually exclusive groupings. In fact, integrating across these groupings will only help to address the complexities of how pollutant exposure affects animals in the wild.
At the individual level, behavioural ecotoxicology research to date has largely focused on how exposure to chemicals alters the average level of ecologically relevant behaviours (e.g. activity, aggression, boldness). Such observations have been pragmatic from a laboratory experimental design perspective but often ignore behavioural variation. This is concerning because phenotypic plasticity, that is an individual’s capacity for phenotypic variation under different environments, is a key determinant of performance under fluctuating conditions within their lifetime (often called ‘acclimation’; see Donelson et al., 2019). Moreover, phenotypic variation among individuals is fundamental for adaptation (and evolution) to occur, meaning that the extent of variation within a population is an important indicator of how buffered that population will be to environmental change, including chemical pollution (Sih, Ferrari & Harris, 2011). Accordingly, the field of behavioural ecology has seen a shift towards quantifying and understanding consistent within- and among-individual behavioural variation (i.e. animal personality; Sih, Bell & Johnson, 2004). This includes both phenotypic plasticity and other sources of behavioural variation such as circadian/circannual rhythms (e.g. Melvin, 2017; Thoré, Brendonck & Pinceel, 2021a), and life-history events (e.g. Thoré et al., 2019). This has produced new insights into how consistent individual behavioural differences are correlated with key traits related to behaviour, such as cognitive ability (Sih & Del Giudice, 2012; Griffin, Guillette & Healy, 2015), metabolic rate (Careau et al., 2008; Biro & Stamps, 2010), and space-use patterns (Spiegel et al., 2017; Michelangeli et al., 2021). Furthermore, individual behavioural variation is heritable (Dochtermann, Schwab & Sih, 2015) but can also be shaped by experience via transgenerational and developmental phenotypic plasticity (Groothuis & Taborsky, 2015), including exposure to chemical pollutants (Tüzün et al., 2017). Importantly, differences in behaviour among individuals may also lead to variation in sensitivity and exposure to chemical pollutants. For example, recent research has demonstrated that social status modulates the extent of uptake of the pharmaceutical pollutant oxazepam by juvenile brown trout (Salmo trutta), with subordinate fish absorbing more of the drug than dominant ones, likely due to higher ventilation and respiration rates (McCallum et al., 2021). Given that only a relatively small number of recent studies have examined how chemical stressors alter aspects of behavioural variation (e.g. Tüzün et al., 2017; Polverino et al., 2021), or how consistent individual differences affect behavioural responses to chemical stressors (Nanninga, Scott & Manica, 2020), the integration of behavioural ecotoxicology and consistent individual variation is clearly an important direction for future study (see Montiglio & Royauté, 2014).

Scaling up to the group and population levels, we know that animals display a range of social phenotypes (Gartland et al., 2021) and that interactions among conspecifics can underlie many aspects of animal fitness (e.g. territory defence, reproduction, parental care). Yet very little attention has been given to understanding how contaminants might affect animal groups and intraspecific interactions.
Table 1. Research in behavioural ecotoxicology has increased markedly in ecological realism, especially in recent years. This includes studies conducted within each of the key areas for future research identified in Fig. 1, although such studies are still relatively rare. Examples of these studies are provided here, grouped by research area.

| Research area           | Compound(s)/stressor(s)                          | Species                   | Major result(s)                                                                 | Reference |
|------------------------|-------------------------------------------------|---------------------------|---------------------------------------------------------------------------------|-----------|
| **Personality**        | Phosmet (organophosphate insecticide)           | Jumping spider (*Eris milensis*) | Repeatability of personality traits declined in the exposed group, mostly mediated by an increase in within-individual variance. | Royauté, Buddle & Vincent (2015) |
| Behavioural differences between individuals that are consistent over time and across contexts (see Montiglio & Royauté, 2014; Jacquin et al., 2020) | Esfenvalerate (pyrethroid insecticide) | Damsel fly (*Carpigion puella*) | Exposure changed average activity and behavioural covariation (activity and boldness) but not behavioural repeatability. | Tužin et al. (2017) |
|                        | Mixture of metals, mainly lead, copper, and zinc | Great tit (*Parus major*)  | Exploration behaviour and aggressiveness during nest defence were repeatable across years. Birds with high levels of lead in their blood and high levels of multiple metals in their feathers exhibited slower exploration behaviour but no effect of exposure was seen on aggressiveness. | Grunst et al. (2019) |
|                        | Fluoxetine (antidepressant pharmaceutical)       | Guppy (*Poecilia reticulata*) | Homogenised individuals’ activity (i.e. reduced consistent variation between individuals). | Tan et al. (2020) |
|                        | Fluoxetine (antidepressant pharmaceutical)       | Guppy (*Poecilia reticulata*) | Homogenised individuals’ activity (i.e. reduced variation between but not within individuals). | Polverino et al. (2021) |
|                        | Chlorpyrifos (organophosphate insecticide)       | Ischnura damselfly species (*I. elegans, I. genei, I. gradisi* and *I. pumilio*) | Exposure affected covariation of life-history and boldness in the most fast-lived species (*I. pumilio*). Fast pace-of-life was associated with higher zinc sensitivity. Zinc exposure made larvae less active, less exploratory, and less risk-taking. Exposure to zinc did not change the covariation patterns between traits (behavioural and physiological). | Debeccker et al. (2016) |
|                        | Zinc (heavy metal)                               | Blue-tailed damselfly (*Ischnura elegans*) | Fluoxetine disrupted sex-specific relationships between body size (proxy for growth rate) and activity. | Debeccker & Stoks (2019) |
|                        | Fluoxetine (antidepressant pharmaceutical)       | Fairy shrimp (*Branchipodopsis wolf*) | Fluoxetine disrupted sex-specific relationships between body size (proxy for growth rate) and activity. | Thoré et al. (2021b) |
| **Pace-of-life syndrome** | 4-nonylphenol (endocrine-disrupting chemical)  | Banded killifish (*Fundulus diaphanus*) | Unexposed fish oriented away from dosed conspecifics. Shoals of all exposed fish had larger nearest-neighbour distances (less-tight shoals). | Ward et al. (2008) |
| A form of social behaviour involving the coordinated behaviour of groups of similar individuals, and the emergent properties of these groups. | Imidacloprid (neonicotinoid insecticide)        | Bumblebee (*Bombus impatiens*) | Impaired nursing behaviour and altered social and spatial dynamics of workers within nests. | Crall et al. (2018) |
|                        | Crude oil                                       | Atlantic croaker (*Micropogonias undulatus*) | Reduced shoal cohesion in shoals with all exposed fish and in shoals with only one exposed fish. | Armstrong et al. (2019) |
|                        | Benzo[a]pyrene (polycyclic aromatic hydrocarbon, PAH) | Zebrafish (*Danio rerio*) | Increased inter-individual distances in exposed shoals. Exposed shoals moved less overall. | Hamilton et al. (2021) |
|                        | Oxazepam (anxiolytic pharmaceutical)             | Brown trout (*Salmo trutta*) | Fish were less aggressive at higher doses and subordinate fish became more competitively successful at low doses (dominant and subordinate fish affected differently). | McGahum et al. (2021) |
| Research area | Compound(s)/stressor(s) | Species | Major result(s) |
|---------------|-------------------------|---------|-----------------|
| Species interactions | | | |
|  | | | |
| Behavioural interactions between individuals of different species, such as predator–prey and competitive interactions (see Saaristo et al., 2018; Fleeger, 2020; Fisher et al., 2021) | Carbaryl (carbamate insecticide), malathion (organophosphate insecticide) | Amphibian prey (gray treefrog, Hyla versicolor; green frog, Rana clamitans; American bullfrog, R. catesbeiana) and a predator (red-spotted newt, Notophthalmus viridescens) | Exposure to either insecticide reduced the activity of all three tadpole prey species, and reduced the predation rate of newts on one tadpole species. |
|  | | | |
| Urban and industrial contamination, including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), dioxins, and metals (e.g. copper, lead, zinc) | Killifish (Fundulus heteroclitus), bluefish (Pomatomus saltatrix), finger crab (Uca pugnax), blue crab (Callinectes sapidus), grass shrimp (Palaemonetes pugio) | At contaminated sites, all five species showed reduced activity and feeding. Complex behavioural changes were also seen within species due to contamination, including reduced predator avoidance in killifish but increased predator avoidance in finger crabs and blue crabs. |
|  | | | |
| Trifl oxide (TFS, strobilurin fungicide) | Eel (Synbranchus marmoratus), four anuran prey species (Rhinella arenarum, Physalaemus santafecinus, Leptodactylus latrans, Elachistocleis bicolor) | Exposure altered the outcome of eel–tadpole interactions by decreasing prey movement and prey detection, increasing non-contact feeding. Eels preyed selectively upon non-exposed tadpoles. Exposure reduced foraging behaviour in both predator species. Exposure to TFS alone reduced prey movement and feeding. Exposure to TFS plus coleopteran predators reduced prey movement and feeding. Exposure to TFS plus carbonate decreased prey movement and feeding. |
|  | | | |
| 17β-oestradiol (E2, oestrogen steroid hormone) | Fathead minnow (Pimephales promelas), bluegill sunfish (Lepomis macrochirus) | Exposure reduced anti-predator escape behaviour of larval minnows and they were more likely to be predated by a sunfish predator. |
|  | | | |
| Dichlorodiphenyltrichloroethane (DDT, organochlorine insecticide) | African clawed frog (Xenopus laevis), mosquito (Culex sp.) | Significant exposure × prey cue interaction. Exposure reduced frog foraging behaviour towards live prey cues, although no effect was seen in response to olfactory prey cues. Mosquito larvae exhibited reduced antipredator behaviour. |
|  | | | |
| Host–parasite interactions | Imidacloprid (neonicotinoid insecticide), entomopathogenic fungi (Metarhizium robertsii and Beauveria bassiana) | Citrus root weevil (Diaprepes abbreviatus) | Application of either fungus had no effect on the movement of larvae in soil, although insecticide exposure was found to impair larval movement. Moreover, exposure to both undiluted and a fungicide in combination with a fungicide increased swimming speed following a simulated predator attack. No singular or interactive effects of parasitic infection were observed. |
|  | | | |
| Chlorpyrifos (organophosphate insecticide), trematode parasite (Euhaplorchis californiensis) | California killifish (Fundulus parvipinnis) | Insecticide exposure reduced swimming speed following a simulated predator attack. Mixtures of metals (cadmium, copper, and zinc) and immune challenge (antigen mixture mimicking a parasite infection) primarily resulted in antagonistic effects on swimming activity. |

Continued...
| Biological Reviews | Sievers et al. (2021)  |
|--------------------|------------------------|
| Table 1.          |                        |

| Research area          | Compound(s)/stressor(s)                                                                 | Species                                           | Major result(s)                                                                                     |
|------------------------|-------------------------------------------------------------------------------------------|---------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| Multiple stressors     | Copper (heavy metal), imidacloprid (insecticide)                                           | Spotted marsh frog (Limnodynastes tasmaniensis)   | Copper increased erratic swimming at the lower imidacloprid concentration. Limited overall evidence for interactive effects. Both stressors produced independent effects in contaminated killifish, and reducing feeding behaviour in normals. |
|                       | Chlorpyrifos (organophosphate insecticide), low-speed temperature                         | California killifish (Fundulus parvipinnis)      | No evidence for interactive effects on reproductive behaviour and prey-patch-scale behaviour being dependent on temperature. |
|                       | Fluoxetine (antidepressant pharmaceutical), acute temperature                              | California killifish (Fundulus parvipinnis)      | Particularly development and transgenerational warming reduced larval antipredator behaviours. Contaminated killifish. |
|                       | Carbamazepine (anticonvulsant pharmaceutical), gemfibrozil (blood lipid-regulating pharmaceutical) | California killifish (Fundulus parvipinnis)      | In the laboratory, fish collected from contaminated ponds exhibited reduced activity and exploration, although this was not reflected in distance moved. |
|                       | Oxazepam (anxiolytic pharmaceutical), European perch (Presetta fluviatilis)                 | European perch (Presetta fluviatilis)             | Promoted downward migratory behaviour in the laboratory and in a natural river tributary. |
|                       | Clothianidin (neonicotinoid insecticide)                                                   | Bumblebee (Bombus terrestris audax)               | In a semi-field experiment, exposure produced notable change in patterns of foraging activity and pollen foraging, with a colony census at the end of the experiment revealing that treated colonies had fewer adults (workers, drones, and gynes) compared to control colonies. |
|                       | Neonicotinoid insecticide mixture                                                         | European honey bee (Apis mellifera)               | Effects on foraging behaviour in unpaved almond crops. Exposure to other drone-rearing male pheromones and pollens increased foraging for nectar. |
|                       | Oxazepam (anxiolytic pharmaceutical), European round goby (Neogobius melanostomus)       | European round goby (Neogobius melanostomus)     | No evidence for interactive effects on reproductive behaviour and prey-patch-scale behaviour being dependent on temperature. |
|                       | Carbamazepine (anticonvulsant pharmaceutical), gemfibrozil (blood lipid-regulating pharmaceutical) | European round goby (Neogobius melanostomus)     | Promoted downward migratory behaviour in the laboratory and in a natural river tributary. |
|                       | Flucloxacillin (antibiotic pharmaceutical), Bumblebee (Bombus terrestris audax)           | Bumblebee (Bombus terrestris audax)               | In a semi-field experiment, exposure produced notable change in patterns of foraging activity and pollen foraging, with a colony census at the end of the experiment revealing that treated colonies had fewer adults (workers, drones, and gynes) compared to control colonies. |
|                       | Carbamazepine (anticonvulsant pharmaceutical), gemfibrozil (blood lipid-regulating pharmaceutical) | Bumblebee (Bombus terrestris audax)               | In a semi-field experiment, exposure produced notable change in patterns of foraging activity and pollen foraging, with a colony census at the end of the experiment revealing that treated colonies had fewer adults (workers, drones, and gynes) compared to control colonies. |
|                       | Bupropion (antidepressant pharmaceutical)                                                  | European honey bee (Apis mellifera)               | Effects on foraging behaviour in unpaved almond crops. Exposure to other drone-rearing male pheromones and pollens increased foraging for nectar. |

© 2022 The Authors. *Biological Reviews* published by John Wiley & Sons Ltd on behalf of Cambridge Philosophical Society.
Table 1. (Cont.)

| Research area | Compound(s)/stressor(s) | Species | Major result(s)                                                                 | Reference |
|---------------|-------------------------|---------|---------------------------------------------------------------------------------|-----------|
| Toxicological, and multigenerational exposures. | Developmental exposure produced hypoactivity and reduced exploratory behaviour in two consecutive generations of unexposed descendants. | Vera-Chang et al. (2019) | Exposure for three generations altered the larval and adult behaviour (foraging, courtship, boldness) of larvae and adults, which were magnified in the F1 and F2 generations. | Müller & Römter (2019); Swank et al. (2003); Müllter (2019) |

(beyond simple dyads). As such, another significant future direction will be to measure the impact of chemical pollutants in natural social settings on endpoints like: (i) social network structure, including the formation and consequences of dominance hierarchies; (ii) collective behaviours such as group shoaling, flocking, foraging, or collective decision-making; (iii) how individual traits (e.g. personality variation) affect group or collective behaviour; (iv) animal contests over valued resources (e.g. territory, mates); and (v) classic reproductive behaviours like courtship, mating and alternative strategies, and parental care. This work can also operate beyond a single generation because an individual’s behaviour can be shaped by both past and current exposure, as well as past conspecific experiences via indirect maternal/paternal effects (Bell & Hellmann, 2019; Donelan et al., 2020).

We also need to look beyond any single species to answer questions concerning multi-species interactions and how behaviour is affected by other ecological processes across environmental gradients. These types of questions will allow us to better approximate the complexities of natural environments and might include, for example, uncovering whether and how contaminants could decouple or change host–parasite or host–pathogen relationships (Blanken, van Langvelde & van Dooremalen, 2015; Rumschlag et al., 2019), alter interspecific information transfer or eavesdropping (Gil et al., 2018), and affect predator–prey interactions (Weis & Candelmo, 2012; Hayden et al., 2015). Moreover, considering that various pollutants (e.g. psychoactive pharmaceuticals) have been shown to alter predator avoidance and escape behaviours in prey species (e.g. Polo-Cavia, Burrago & Gomez-Mestre, 2016; Martin et al., 2017), key questions that have received surprisingly little attention to date are whether exposure of predators and/or prey to (the same or different) contaminants might shift the “landscape of fear” (Gaynor et al., 2019), or shape animal communities and trophic cascades through altered patterns of competition and/or consumption (e.g. Rohr, Kerby & Sih, 2006; Weis et al., 2011).

Finally, to reveal patterns of effects of chemicals on individuals, animal groups, and whole community/ecosystem outcomes, a crucial need is to understand the relative impact of chemical stressors and other abiotic and biotic stressors that can have unexpected and complex effects on animal behaviour, that is we need a multi-stressor approach (Orr et al., 2020). Numerous studies show that even very low levels of chemical stressors can severely reduce fitness if combined with low food levels (e.g. Rohr et al., 2004), predation risk (Relyea, 2003), high parasite load (e.g. Rumschlag et al., 2019), or variable temperature (e.g. Brown et al., 2015; Delnat et al., 2019). Yet relatively few studies have examined how behaviour might mediate these multi-stressor outcomes via animal dispersal or avoidance, seasonal or diurnal shifts in behaviour, or increasing/decreasing behaviours to compensate for changing metabolic demands (e.g. a foraging–detoxification trade-off). Among species, some of the variation in negative impacts of chemical stressors will be due to differences in physiology that underlie inherent
differences in vulnerability to exposure. However, differences in behavioural responses will likely play a major role in explaining dissimilar exposure to chemicals, and in the ability of animals to compensate behaviourally, for example by increasing energy intake or via social buffering. Thus, more accurate behavioural assessments of chemical pollutants will often require a multi-stressor approach in order to assess the simultaneous or sequential effects of chemicals with other key environmental factors within ecologically relevant conditions.

IV TOOLS AND TECHNOLOGIES FOR ADVANCING BEHAVIOURAL ECOTOXICOLOGY

Here, we provide an overview of recent methodological and technological innovations that allow for the possible effects of contaminant exposure on organismal behaviour to be investigated at a greater resolution and scale than ever before (Fig. 2). See the online Supporting Information for a comprehensive overview of suppliers and resources for laboratory software and hardware (Tables S1 and S2, respectively), and field software and hardware (Tables S3 and S4).

(1) Laboratory-based experimental innovations

There is now a tremendous variety of laboratory software and hardware options available to ecotoxicologists for capturing potential behavioural responses to contaminant exposure. For instance, cameras with high frame rates offer the temporal resolution to track behaviours that were previously not possible to capture. This includes recording high-speed locomotion on land (e.g. walking parameters of insects; Pfef-fer et al., 2019), in water (e.g. propulsion in swimming fish; Johansen et al., 2017), and in the air (e.g. honeybee flight paths; Guiraud, Roper & Chittka, 2018), as well as feeding performance of predators (Rossoni & Niven, 2020), and escape performance of prey (e.g. Jonsson et al., 2019). Similarly, cameras with wide spectral- or thermal-sensing...
capabilities enable researchers to record behaviours in the dark (e.g. Mitchell & Clarke, 2019), while multi-camera systems track animal movement in three-dimensional space (Macrì et al., 2017). Regarding high-throughput approaches, lab-on-a-chip devices, which make use of the changes in laminar flow within a chamber, have been developed for an array of aquatic organisms (Campana & Włodkowic, 2018). These small chips are versatile to a variety of sensors and can measure behaviour following or during chemical exposure, in binary conditions as well as complex chemical gradients (Campana & Włodkowic, 2018).

In addition to monitoring behaviours of pre-exposed organisms or those exposed in real time, many new technologies have the ability to stimulate behaviours using light, vibration/noise, or electrical signals (discussed in Ågerstrand et al., 2020). This allows for the measurement of behavioural traits such as reaction time and speed, turning angles, space use, and predator avoidance. More sophisticated technologies are also being adapted to stimulate social, cognitive, and learning behaviours. For instance, state-of-the-art robots can now be used to interact with fish in real time to study the underpinnings of social and anxiety-related behaviours (e.g. Polverino et al., 2019), and even reveal the long-lasting consequences of brief behavioural alterations on the life-history, reproduction, and ecological success of targeted animals (Polverino et al., 2022). Indeed, despite being underexploited in behavioural ecotoxicology to date, bioinspired robots offer an autonomous, customisable, and repeatable approach that overcomes challenges associated with more traditional methods, in which live stimuli are an extra source of variation, although at the cost of some degree of biological and ecological realism. In addition to visual stimuli, new approaches also allow for increasingly sophisticated analysis of behavioural responses to chemical signals. For example, wind-tunnel assays are allowing researchers to determine the preference of pollinating insects for non-altered versus ozone-contaminated floral blends (Cook et al., 2020), while two-current choice flumes are increasingly used to examine chemosensory function in aquatic species (see Jutfelt et al., 2017), including attraction towards or avoidance of contaminants (Horký et al., 2021).

In parallel, automated visual tracking is fast becoming an indispensable tool for quantifying animal behaviour (reviewed in Henry & Włodkowic, 2020; Panadeiro et al., 2021). There is now a wide variety of programs available for tracking animal movement, including a number of recently developed open-source systems, such as TRex (Fig. 2A, Walter & Couzin, 2021) and ToxTrac (Rodriguez et al., 2018). Both of these systems are fast, powerful, user-friendly, and versatile across a wide range of model species. Furthermore, these programs represent a major advance given their capacity to track large groups of animals simultaneously, for example ≤1000 individuals using TRex, including deep-learning-based visual identification of up to 100 unmarked individuals (Walter & Couzin, 2021). This provides an unparalleled opportunity to study the potential effects of contaminants on complex social behaviours in animal groups, such as dominance hierarchies and collective motion. Moreover, although distinctly underutilised in ecotoxicology to date, newly available software packages [e.g. DeepPoseKit (Graving et al., 2019); TRex (Walter & Couzin, 2021)] also allow for the quantification of an array of behavioural measures in addition to positional data, such as stereotyped movements (e.g. courtship displays), visual field (Fig. 2B), and posture analysis (e.g. body shape, head/tail position; Fig. 2C). As alluded to above, the integration of artificial intelligence and machine learning algorithms into these systems continues to power a revolution in motion-tracking technology, including using deep learning to identify specific animals, determine posture, and even detect behaviours that were previously invisible to human observers (discussed in Graving et al., 2019).

From a mechanistic perspective, an increasing number of methods in molecular neurotoxicology are now available for uncovering drivers of pollutant-induced behavioural changes (reviewed in Bownik & Włodkowic, 2021). These methods range from transcriptomics (i.e. gene transcript analysis) to proteomics (i.e. analysis of proteins) and enzymatic assays, metabolomics (i.e. analysis of chemical processes involving metabolites in cells and tissues), and examination of sub-cellular processes such as programmed cell death (Bownik & Włodkowic, 2021). An important recent advance that promises to increase mechanistic understanding in behavioural ecotoxicology is large-scale neurotransmitter profiling, enabled by the above-mentioned rapid developments in analytical chemistry (see Section II.1). Here, an array of neurotransmitters can be monitored, as well as their precursors and degradation products, across different brain regions, providing a direct mechanistic link between pollutant-induced behavioural changes and altered neuronal transmission (Mayol-Cabrè et al., 2020). For example, pioneering research harnessing neurotransmitter profiling recently demonstrated direct effects of the pervasive herbicide glyphosate on fish monoaminergic systems, with resulting effects on oxidative stress and anxiety-related behaviours (Faria et al., 2021). Furthermore, significant recent breakthroughs mean that we can now dissect neural mechanisms for chemically induced brain activation and disruption using genetically encoded (bio)sensor systems coupled with high-speed fluorescence light-sheet microscopy (LSM; Winter et al., 2017; Fig. 2D). Illustrating this, transgenic zebrafish (Danio rerio) with pan-neuronal expression of the calcium indicator GcaMP6, coupled with LSM, allow for functional imaging with sub-cellular resolution across the whole brain, which has been applied to uncover mechanisms associated with behavioural responses to drug treatments (Winter et al., 2021).

**Scaling up: experimental advances in the field**

When assessing possible impacts of contaminants on behaviour, a holistic approach is ideal, that is studies relating mechanistic explanations to experiments spanning laboratory, semi-field, and field contexts. While laboratory assays

*Biological Reviews* 97 (2022) 1346–1364 © 2022 The Authors. *Biological Reviews* published by John Wiley & Sons Ltd on behalf of Cambridge Philosophical Society.
provide valuable insights in a standardised setting and can be important for validating field observations (both of which are fundamental to ecotoxicity studies to support various tiers of chemical risk assessments), semi-field- and field-based ecotoxicology is crucial for understanding the potential impacts of contaminants in large-scale, complex, and dynamic natural systems. Encouragingly, experimental and technological innovations continue to emerge that allow for effects of chemical pollution on animal behaviour to be measured in the field with ever-greater efficiency and accuracy. This includes advances in remote-sensing technology, which have revolutionised our capacity to gather high-resolution behavioural and physiological data from animals in the wild (Smith & Pinter-Wollman, 2021; Nathan et al., 2022). Cheaper, smaller, and increasingly more capable transmitters and sensors allow researchers to tag and track animals over large spatial and temporal scales (Smith & Pinter-Wollman, 2021; Nathan et al., 2022). For example, automated barcode systems enable whole-group or even whole-population tracking over months or potentially years (Alarcón-Nieto et al., 2018; Fig. 2E). Meanwhile, radio-frequency identification (RFID) tagging has revealed changes in the lifetime foraging behaviour of free-ranging honeybees exposed to a neonicotinoid pesticide (Colin et al., 2019; Fig. 2F), and Global Location Sensors (GLS) have been used to study spatial and seasonal trends of mercury contamination in seabirds from Arctic breeding colonies (Renedo et al., 2020; Fig. 2G).

Perhaps the most transformative use of remote-sensing technology can be found in aquatic environments, where tools such as acoustic telemetry are leapfrogging our understanding of the behaviour and ecology of aquatic organisms by enabling detailed spatial monitoring of individuals over whole freshwater systems (e.g. rivers and lakes; Fig. 2H) and even oceans (reviewed in Hellström et al., 2016b). Data previously difficult or impossible to obtain from the field, such as survival, schooling behaviour, home ranges, diel activity patterns, and predator–prey interactions can now be investigated in the wild at a level of detail comparable to that of laboratory studies (Hellström et al., 2016b). Further, we anticipate that future research pairing acoustic telemetry with newly developed methods of remote contaminant exposure (e.g. slow-release implants; McCallum et al., 2019) and/or spatial contaminant modelling (Fonseca et al., 2020) will provide valuable insights into the real-world behavioural impacts of chemical pollution. Parallel to the progress made in animal tracking technology, the ‘golden age of bio-logging’ is providing an ever-expanding variety of small physiological and behavioural sensors that can record everything from an animal’s heart rate, body temperature, and acceleration, to detailed data on foraging and spawning behaviour (reviewed in Whitford & Klimley, 2019). In combination, these advances in remote-sensing technology provide a powerful, yet largely unexplored, experimental toolkit to study impacts of pollution on behavioural and ecological processes. Harnessing these novel approaches will also enable research attempting to establish a causal chain from pollutant-induced changes in individual- and group-level behavioural variation to population responses and ecosystem change. Still, given the complexity of natural environments, it is important to note that careful experimental design (including the use of suitable controls), and appropriate statistical and biological interpretation of collected data, will be critical to the validity and reliability of such studies.

V IMPROVING STATISTICAL SOPHISTICATION

Historically, ecotoxicology experiments for use in chemical risk assessment follow strict regulatory guidelines (Chapman et al., 1996). These guidelines have generally employed simple experimental designs and straightforward statistical analyses to maximise external validity and ensure protocols are broadly accomplishable by researchers (Chapman et al., 1996). However, ever-increasing environmental and ecological complexity is being incorporated into behavioural ecotoxicology studies (see Sections II and III, respectively), and new technologies have changed the nature of the data extracted from these experiments (see Section IV). As a result, various statistical techniques conventionally used in ecotoxicology may not always be appropriate for use in modern behavioural ecotoxicology.

(1) Embracing mixed modelling to address biological complexity

Biological data, and particularly behavioural endpoints, are often inherently variable, unbalanced (i.e. unequal sample sizes for different classes), non-normally distributed, and highly structured (i.e. containing groups of non-independent units). Such data sets require statistical approaches that are capable of appropriately partitioning and quantifying behavioural variation, while also considering the hierarchical nature of the data (i.e. to avoid pseudoreplication and/or to deal with non-independence). One increasingly popular approach is mixed-effects modelling (i.e. multilevel or hierarchical modelling) of either normally distributed response variables (linear mixed model, LMM) or non-normally distributed response variables, such as count, binary or proportion data (generalised linear mixed models, GLMMs). Mixed-effects models are a sophisticated statistical tool that can be applied to analyse data that have both fixed effects (e.g. experimental treatment, sex) and one or more clustering (random) factors (e.g. individual ID, treatment replicate) (Bolker et al., 2009). Mixed models can therefore account for, and estimate, the variation contributed by hierarchical structures in the data. A common example of this is the use of mixed models to account for multiple observations made on the same individual, where individual IDs are included in the random structure of the model as random intercepts to account for, and estimate, the amount of variation within and among individuals. This capability has meant that mixed models are now widely used in behavioural ecology and
evolutionary biology to partition the variance across response variables both within and across grouping factors, and reveal effects occurring at each level (Dingemanse & Dochtermann, 2015). As previously discussed in this review, inferring the magnitude of variation within and among groups can be highly informative in the context of understanding responses to chemical pollution, which is now made possible via mixed-modelling approaches, including using movement data in the wild (Hertel et al. 2020). Mixed models can also accommodate multiple response variables simultaneously (i.e. multivariate mixed models), allowing for the direct estimation of both within- and between-individual phenotypic (co)variances. Further, particularly relevant for complex experimental designs, data-reduction techniques (e.g. principal component analysis) can be used in combination with mixed modelling to collapse multiple related variables into fewer uncorrelated predictors in order to avoid over-parameterisation. Fortunately, many publications on mixed models include detailed step-by-step guides on applying such statistical approaches to answer specific biological questions (summarised in Table S5).

(2) Harnessing meta-analytic approaches

We would also like to highlight the scarcity of meta-analytic studies in the field of behavioural ecotoxicology. This is surprising given that sufficient data exist addressing behavioural effects of chemical pollutants to answer targeted meta-analytic hypotheses/questions. Beyond this general call for meta-analyses, we also suggest that behavioural ecotoxicologists draw from the recent advances in meta-analytic methods developed in the fields of ecology and evolution (Nakagawa et al., 2017). For example, future meta-analyses could focus on the impacts of chemical pollution on behavioural variance (i.e. total phenotypic variance of unexposed versus exposed populations), in parallel with a more ‘traditional’ mean-focused meta-analysis (Nakagawa et al., 2015). More specifically, in addition to comparing the means across different treatment groups of interest using standardised metrics such as Cohen’s $d$, Hedges’ $g$, or the response ratio, the natural logarithm of the ratio between the coefficients of variation (lnCVR) can also be extracted, allowing for meta-analytical comparisons of differences between the variability in groups (for a detailed description, see Nakagawa et al., 2015). In addition, for broad research questions in behavioural ecotoxicology, systematic mapping will be beneficial to identify areas that require more research and those that are ready for meta-analytic synthesis (see James, Randall & Haddaway, 2016), despite being currently underused in ecotoxicology (Wolff et al., 2019). Further, to gain a more nuanced understanding of the structure of a research topic, a novel meta-analytic technique called ‘research weaving’ could be used, which combines systematic mapping and bibliometrics (see Nakagawa et al., 2019).

VI CONCLUSIONS

(1) Behavioural studies represent a sensitive, powerful, and ecologically meaningful approach for assessing the effects of environmental contaminants.

(2) As research in this area continues to grow and increase in complexity, we emphasise the importance of following basic principles of sound ecotoxicology wherever possible (Harris et al., 2014), for example using appropriate exposure route(s), testing a suitable number and span of exposure treatments, and quantifying exposure concentrations. Although not all principles apply to all studies, and certain principles can be challenging or impossible to implement in some cases (e.g. in large-scale or long-term studies), they are especially vital if researchers intend their work to be translationally applicable to chemicals risk assessment and/or regulation (Ågerstrand et al., 2020).

(3) We encourage researchers working within behavioural ecotoxicology and related disciplines to explore the rich toolkit that is now available to propel our understanding of how chemical pollution impacts wildlife living in a rapidly changing world.

VII. ACKNOWLEDGEMENTS

Support for this review was provided by a Swedish Research Council Formas Mobility Grant (2020-02293 to M.G.B.), the Kempe Foundations (SMK-1954 and SMK21-0069 to M.G.B.), the Marie-Claire Cronstedt Foundation (to M.G.B.), the ÅFors Foundation (20-51 to M.G.B.), the National Institute of Environmental Health Sciences (1P01ES028942 to B.W.B.), the EU Cross-Channel Interreg Programme (RedPol; Interreg 5a #183 to A.T.F.), the Forrest Research Foundation (Forrest Fellowship to G.P.), an Australian Government Research Training Program (RTP) Scholarship (to J.A.B. and H.T.), the Australian Research Council (DP190100642 to B.B.M.W, and L.A.A., and DP220100245 and FT190100014 to B.B.M.W), and the Swedish Research Council Formas (2020-00961 to E.S.M., and 2018-00628 to T.B.).

VIII. REFERENCES

References marked with an asterisk (*) are cited in the online Supporting Information. Agerstrand, M., Arnold, K., Balshine, S., Bredin, T., Brooks, B. W., Maack, G., McCallum, E. S., Pyle, G., Saaristo, M. & Ford, A. T. (2020). Emerging investigator series: use of behavioural endpoints in the regulation of chemicals. Environmental Science: Processes & Impacts 22, 49–65.

Alarcón-Nieto, G., Graiving, J. M., Klarevan-Irby, J. A., Maldonado-Chaparro, A. A., Mueller, I. & Farine, D. R. (2018). An automated barcode tracking system for behavioural studies in birds. Methods in Ecology and Evolution 9, 1530–1547.

Allegue, H., Araujo-Ajoy, Y. G., Dingemanse, N. J., Dochtermann, N. A., Garamszegi, L. Z., Nakagawa, S., Réale, D., Schielzeth, H. & Westneat, D. F. (2017). Statistical quantification of individual differences (SQuID): an educational and statistical tool for understanding multilevel phenotypic data in linear mixed models. Methods in Ecology and Evolution 8, 257–267.
Ankley, G. T., Bennett, R. S., Erickson, R. J., Hoff, D. J., Hornek, M. W., Johnson, R. D., Mount, D. R., Nichols, J. W., Russom, C. L., Schmieder, P. K., Serrano, J. A., Tietje, J. E. & Villenueve, D. L. (2010). Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environmental Toxicology and Chemistry 29, 730–741.

Araújo, C. V., Liaisagui, A., Silva, D. C., Ramos-Rodríguez, E., González-Ortegón, E., Espindola, E. L., Baldó, F., Mena, F., Parrá, G., Blanco, J., López-Doval, J., Sendra, M., Banni, M., Islam, M. A. & Moreno-Garrido, J. (2020). Not only toxic but repellent: what can organisms’ responses tell us about contamination and what are the ecological consequences when they flee from an environment? Toxics 8, 118.

Arce, A. N., David, T. I., Randall, E. L., Ramos Rodrigues, A., Colgan, T. J., Wurm, Y. & Gill, R. J. (2017). Impact of controlled necrotomous exposure on burn heath in a larval field setting. Frontiers in Ecology and Evolution 54, 1199–1208.

Armstrong, T. S., Khursigara, A. J., Killen, S. S., Fearney, H., Parsons, K. J. & Esbaugh, A. J. (2019). Oil exposure alters social group cohesion in fish. Scientific Reports 9, 13520.

Asaadpour, M. & Beyan, C. (2015). A computer-assisted system for photographic mark-recapture and risk assessment. Environmental Toxicology and Chemistry 34, 169–173.

Bakker, J. D., Oppenheimer, R. L., van Beukering, P. K. & Buikema, D. (2013). Predicting animal behaviour using deep learning: GPS data alone accurately predict diving in seabirds. Methods in Ecology and Evolution 9, 681–692.

Van der Heijden, S., Veerman, E., Franken, J. M., Corbey, J. M., Morsø, T. & Goethals, P. (2020). Quantifying and reducing epistemic uncertainty of passive acoustic telemetry data from longitudinal aquatic systems. Ecological Informatics 59, 101133.

Bennet, B. C. & Grue, E. M. (2017). Brms: An R package for Bayesian multilevel models using Stan. R Journal 10, 395–411.

Bennik, K., van Lokhorst, J., Nollet, I., den Boer, P., Das, J. & Biswas, R. (2016). An intelligent positioning system for birds. Environmental Toxicology and Chemistry 35, 1835–1842.

Berg, C. W., Johnson, E. S., Johnston, M., Wall, A. M. & Tyler, C. R. (2015). Climate change and pollution speed declines in zebra finch populations. Proceedings of the National Academy of Sciences of the United States of America 112, 1237–1246.

Browning, E., Bolton, M., Owen, E., Shoji, A., Gullford, T. & Freeman, R. (2018). Predicting animal behaviour using deep learning: GPS data alone accurately predict diving in seabirds. Methods in Ecology and Evolution 9, 681–692.

Burton, A. C., Nakagawa, S., Huyvaert, K. P. (2011). AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. Behavioral Ecology and Sociobiology 65, 23–35.

Campana, O. & Wlodarczyk, D. (2018). Ecotoxicology goes on a chip: embracing miniaturized bioanalysis in aquatic risk assessment. Environmental Science & Technology 52, 932–946.

Carrillo, D., Walker, S. C., Gill, R. J., Brodin, T., Gjelland, K. Ø., Backhaus, T., Mount, D. R., Nichols, J. W., Russom, C. L., Schmieder, P. K., Serrano, J. A., Khursigara, A. J., Ramos Rodrigues, A., Corbey, J. M., Morsø, T. & Goethals, P. (2020). Quantifying and reducing epistemic uncertainty of passive acoustic telemetry data from longitudinal aquatic systems. Ecological Informatics 59, 101133.

Carbone, D., Colacino, S., Marrella, M. & Réale, D. (2008). Energy metabolism and animal personality. Oikos 117, 641–653.

Carlip, R. E. & Collier, A. L. (2015). Invasive species: an international laboratorium for understanding the mechanisms and multigenerational consequences of transgenerational plasticity. Annual Review of Ecology, Evolution, and Systematics 50, 97–118.

Berg, C. W., Johnson, E. S., Johnston, M., Wall, A. M. & Tyler, C. R. (2015). Climate change and pollution speed declines in zebra finch populations. Proceedings of the National Academy of Sciences of the United States of America 112, 1237–1246.

Browning, E., Bolton, M., Owen, E., Shoji, A., Gullford, T. & Freeman, R. (2018). Predicting animal behaviour using deep learning: GPS data alone accurately predict diving in seabirds. Methods in Ecology and Evolution 9, 681–692.

Brunner, S., Verhees, F. M., Heemstra, J. M., Corbey, J. M., Morsø, T. & Goethals, P. (2020). Quantifying and reducing epistemic uncertainty of passive acoustic telemetry data from longitudinal aquatic systems. Ecological Informatics 59, 101133.

Brunner, B. C. (2017). Brms: An R package for Bayesian multilevel models using Stan. R Journal 10, 395–411.

Burren, K. P., Anderson, D. R. & Huyvaert, K. P. (2011). AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. Behavioral Ecology and Sociobiology 65, 23–35.

Camps, O. & Wlodarczyk, D. (2018). Ecotoxicology goes on a chip: embracing miniaturized bioanalysis in aquatic risk assessment. Environmental Science & Technology 52, 932–946.

Carragiani, A., Banks, P. B., Burton, A. C., Finlay, C. M., Haswell, P. M., Hayward, M. W., Rowcliffe, M. J. & Wood, M. D. (2017). A review of camera trapping for conservation behaviour research. Remote Sensing in Ecology and Conservation 3, 109–122.

Carleu, V., Thomas, D., Humphries, M. & Réale, D. (2008). Energy metabolism and animal personality. Oikos 117, 641–653.

Carriere, E., Nogueida, D. & Wlodarczyk, D. (2015). GammaMucChip:innovative chip-based technology for understanding the marine amphipod Allisochetus compressa. Proceedings of SPIE, Bio-MEMS and Medical Microsystems IF8518, 9351U2.

Chapman, P. M., Crane, M., Wiles, J., Noppert, F. & McIndoe, E. (1996). Improving the quality of statistics in regulatory toxicology tests. Ecotoxicology 5, 167–186.

Chen, S., Xu, H., Wu, J., Yue, R., Yuan, C. & Wang, L. (2019). Deer crossing road detection with roadside LiDAR sensor. IEEE Access 7, 65944–65954.

Cheng, Y., Fielder, W., Wienski, M. & Flack, A. (2019). “Closer-to-home” strategy benefits juvenile survival in a long-distance migratory bird. Ecology and Evolution 9, 8945–8952.

Christin, S., Hervert, É. & Lenormand, N. (2019). Applications for deep-learning in ecology. Methods in Ecology and Evolution 10, 1632–1644.

Cleasby, T. R., Nagakawa, S., Schielzeth, H. & Hafidi, J. (2015). Quantifying the predictability of behaviour: statistical approaches for the study of between-individual variation in the within-individual variance. Methods in Ecology and Evolution 6, 27–37.

Colin, T., Meikle, W. G., Wu, X. & Bannor, A. B. (2019). Traces of a neonictinoid induce precocious foraging and reduce foraging performance in honey bees. Environmental Science & Technology 53, 8252–8261.

Colin, T., Forster, C. G., Westcott, J. W., Xu, X., Meikle, W. G. & Bannor, A. B. (2021). Effects of late mateic treatments on foraging and colony productivity of European honey bees (Apis mellifera). Apidologie 52, 1–19.

Colot, A., Caprari, G. & Greiwalt, R. (2004). ImBot: design of an autonomous miniature mobile robot able to interact with cockroaches. IEEE International Conference on Robotics and Automation 2418–2423.

Cook, B., Haverkamp, A., Hansson, B. S., Roulston, T., Lerda, M. & Knaden, M. (2020). Pollination in the Anthropocene: a moth can learn ozone-altered floral blends. Journal of Chemical Ecology 46, 897–906.

Cooke, S. J., Midwood, J. D., Them, J. D., Kelm, P., Lucas, M. C., Thorstad, E. B., Epler, J., Holbrook, C. & Eiser, B. C. (2013). Tracking animals in freshwater with electronic tags: past, present and future. Marine and Freshwater Research 64, 1185–1190.

Crangi, R., Gravish, N., Mountcastle, A. M. & Combes, S. A. (2015). BEEtag: a low-cost, image-based tracking system for the study of animal behavior and locomotion. PLoS One 10, e0136487.

Crangi, J. D., Switzer, C. M., Oppenheimer, R. L., Versyp, A. N., Dew, B., Brown, A., Eyster, M., Guérin, C., Pierce, N. E., Combes, S. A. & de
Frontiers in quantifying wildlife behavioural responses

Hellström, G.
Hayden, M. T.
Hebert, L.
Hadfield, J. D.
Hill, A. P.

(2016). Implantation and recovery of long-term archival transceivers in 

Inger, R.

Movement Ecology – 129

complex impact of the human-altered sensory environment on animal perception 

responses to multiple stressors and multiple stimuli. 

Martinoli, A.

Upscaling behavioural studies to the

Hups, N. E., Kessel, S. T., Aarestrup, K., Cooke, S. J., Cowley, P. D., Fink, A. T., Harcourt, R. G., Holland, K. N., Iverson, S. J., Kocire, J. F. & Flemming, J. E. M. (2015). Aquatic animal telemetry: a panoramic window into the freshwater world. 

Jackson, B. E., Evangelista, D. J., Ray, D. D. & Hedrick, T. L. (2016). 3D for the people: multi-camera motion capture in the field with consumer-grade cameras and open source software. Biology Open 5, 1334–1342.

Jaccquin, L., Petitjean, Q., Côté, J., Lafaille, P. & Jean, S. (2020). Effects of pollution on fish behavior, personality, and cognition: some research perspectives. Frontiers in Ecology and Evolution 8, 86.

James, K. L., Randall, N. P. & Haddaway, N. R. (2016). A methodology for systematic mapping in environmental sciences. Environmental Evidence 5, 7.

Javer, A., Currey, M., Lee, G. W., Horakson, J. L., Li, K., Martineau, C. N., Yenai, M. P., Bouker, B. M. & McCoy, C. A. (2019). High-speed imaging reveals how anastomous habitat escape changes in aquatic insect prey. Science of the Total Environment 648, 1257–1262.

Joo, R., Boone, M. E., Clay, T. A., Patrick, S. C., Cluessa-Trullas, S. & Basille, M. (2020). Navigating through the R packages for movement. Journal of Animal Biology 89, 248–267.

Junges, C. M., Peltzer, P. M., Lajmanovich, R. C., Attademo, A. M., Zeng, M. C. & Basille, M. (2017). Toxicity of the fungicide trifluthion to tadpoles and its effect on fish-tadpole interaction. Chemosphere 179, 1348–1354.

Jutfelt, F., Sundin, J., Raby, G. D., Krang, A. S. & Clark, T. D. (2017). Two-current choice flumes for testing avoidance and preference in aquatic animals. Methods in Ecology and Evolution 6, 379–390.

Juškaitis, R. G., Helgen, H. W., Brooks, B. W., Duda, K., Slavěk, O., Hureňa, P., Sanchez Santos, E. M. & Randák, T. (2021). Methamphetamine pollution elicits addiction in wild fish. Journal of Experimental Biology 224, jeb241145.

Houssy, T. & Wilcox, A. (2017). Avoiding the misuse of BLUP in behavioral ecology. Behavioural Ecology 28, 948–952.

Hay, R., Piggott, J. J. & Weaver, S. E. (2017). Describing and understanding behavioral responses to multiple stresses in larval multiple insects. Ecology and Evolution 7, 38–17.
**Nakagawa, S. & Schielzeth, H.** (2013). A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods in Ecology and Evolution* 4, 133–142.

**Nakagawa, S., Pullin, R., Mengerink, K., Reinhold, K., Engqvist, L., Lagisz, M. & Senior, A. M.** (2015). Meta-analysis of variation: ecological and evolutionary applications and beyond. *Methods in Ecology and Evolution* 6, 143–152.

**Nakagawa, S., Johnson, P. C. D. & Schielzeth, H.** (2017). The coefficient of determination R² and intraspecific correlation coefficient from generalized linear mixed-effects models revised and expanded. *Journal of the Royal Society Interface* 14, 20170213.

**Nakagawa, S., Noble, D. W. A., Senior, A. M. & Lagisz, M.** (2017). Meta-evaluation of meta-analytic ten appraisal questions for biologists. *BMC Biology* 15, 18.

**Nanwadi, S., Samarasinghe, G., Hadidawar, N. R., Westgate, M. J., O’Dea, R. E., Noble, D. W. A. & Lagisz, M.** (2019). Research weaving: visualizing the future of research synthesis. *Trends in Ecology & Evolution* 34, 224–238.

**Nanninga, G. B., Scott, A. & Manica, A.** (2020). Microplastic ingestion rates are phenotype-dependent in juvenile anemones. *Environmental Pollution* 259, 113635.

**Nakagawa, M. G. Bertram et al.** (in press). Quick guide for Lolitrack. *Loligo-Systems* 1362.

**Michael G. Bertram et al.** (2021). Performance: An R package for assessment, comparison and testing of statistical models. *Journal of Open Source Software* 6, 3139.

**Linnell, J. D. C., McCallum, E. S., Melvin, S. D., McCord, J. P., Kingsford, R. T. & Callaghan, C. T.** (2010). Monitoring large and complex wildlife aggregations with drones. *Methods in Ecology and Evolution* 10, 1024–1035.

**Magnusson, A., Skaug, H., Nielsen, A., Berg, C., Kristensen, K., Maechler, M., Van, Benthem, K., Bolker, B., Brooks, M. & technology, science, and application. *Biometrics* 67, 884–896.

**Lin, Y., Hsiung, J., Piersall, R., White, C., Lowe, G. G. & Clarke, C. M.** (2017). A multi-autonomous underwater vehicle system for autonomous tracking of marine life. *Journal of Field Robotics* 34, 757–774.

**Loligo-Systems (in press).** Quick guide for Lolitrack. 1, 5–4.

**Landecker, D., Ben-Shachar, M. S., Pati, I., Waggoner, P. & Marowski, D.** (2021). Three-dimensional scoring of seabird behaviour unveiled biological phenomena hidden by two-dimensional analyses. *Scientific Reports* 7, 1962.

**Marenette, J. R., Tong, S., Wang, G., Sopinka, N. M., Taves, M. D., Koons, M. & Milhous.** (2017). Behavior as biomarker? Laboratory versus field movement in round goby (Neogobius melanostomus) from highly contaminated habitats. *Ecotoxicology* 26, 1003–1012.

**Martin, J. M., Saaristo, M., Bertram, M. G., Lewis, P. J., Coggan, T. L., Clarke, B. O. & Wong, B. B. M.** (2017). The psychoactive pollutant fluoxetine compromises antipredator behaviour in fish. *Environmental Pollution* 222, 592–599.

**Martin, J. M., Saaristo, M., Tan, H., Bertram, M. G., Nagarajan-Radhya, V., Dowling, D. K. & Wong, B. B. M.** (2019). Field-realistic antidepressant exposure disrupts group foraging dynamics in mosquitofish. *Biological Letters* 15, 20190615.

**Martin, J. M. & McCullum, E. S.** (2021). Incorporating animal social context in ecotoxicology: can a single individual tell the collective story? *Environmental Science & Technology* 55, 10908–10910.

**Manon, R. T., Martin, J. M., Tan, H., Brand, J. A., Bertram, M. G., Tingley, R., Todd-Weickmann, A. & Wong, B. B. M.** (2021). Context is key: sensitivity of individual Arctic charr to seabird predation and its effects on shoaling behavior in fish. *Environmental Science & Technology* 55, 13024–13032.

**Mayol-Carrè, M., Prats, E., Raldúa, D. & Gómez-Canella, C.** (2020). Characterization of monoamine neurotransmitters in the different brain regions of adult zebrafish. *Science of the Total Environment* 743, 114205.

**McCullum, E. S., Cerveny, D., Fick, J. & Brodie, T.** (2019). Show-release implants for manipulating contaminant exposures in aquatic wildlife: a new tool for field ecotoxicology. *Environmental Science & Technology* 53, 8282–8290.

**McCullum, E. S., Dev, C. J., Cerveny, D., Bone, A. P. H. & Brodin, T.** (2021). Social status modulates the behavioral and physiological consequences of a chemical pollution in two groups. *Ecological Applications* 31, e02434.

**McCord, J. P., Groff, L. C. & Sobol, J. R.** (2022). Quantitative non-targeted analysis bridging the gap between contaminant discovery and risk characterization. *Environmental International* 138, 107011.

**Mejering, E., Devezichky, O. & Smal, I.** (2012). Methods for cell and particle tracking. *Methods* 54, 186–200.

**Mevel, S. D.** (2017). Effect of antidepressants on circadian rhythms in fish: insights and implications regarding the design of behavioural toxicity tests. *Aquatic Toxicology* 182, 20–30.

**Mevel, S. D. & Wilson, S. P.** (2013). The utility of behavioral studies for aquatic toxicology: a meta-analysis. *Chemosphere* 93, 2217–2223.

**Meng, S., Tran, T. T., Delnay, V. & Stoks, R.** (2021). Transgenerational exposure to warming reduces the sensitivity to a pesticide under warming. *Environmental Pollution* 284, 112717.

**Michaelangelii, M. Z., Payne, E., Speigel, O., Senn, D., Liu, S. T., Gartner, M. & Shi, A.** (2021). Personality, spatiotemporal ecological variation, and resident/explorer movement syndromes in the sleepy lizard. *Journal of Animal Ecology* 91, 210–223.

**Mitchell, W. F. & Clarke, R. H.** (2019). Using infrared thermography to detect night-roosting birds. *Journal of Field Ornithology* 90, 39–51.

**Montgoy, P. & Royauté, R.** (2014). Concomitants as a neglected source of behavioural variation. *Animal Behaviour* 88, 29–35.

**Müller, T., Römer, C. I. & Müller, C.** (2019). Parental sublethal insecticide exposure prolongs mating response and decreases reproductive output in offspring. *Journal of Applied Ecology* 56, 1528–1537.

**Munson, A. M., Calhoun, W. M. & Shi, A.** (2021). Stable social groups foster conformity and among-group differences. *Animal Behaviour* 174, 197–206.

**Nakagawa, S. & Schielzeth, H.** (2010). Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biological Reviews* 85, 935–956.
Renedo, M., Relyea, R. A., Quintela, E., Pyle, G., Rumschlag, S. L., Polverino, G., Renick, V. C., Raies, A. B., Frontiers in quantifying wildlife behavioural responses to sublethal pesticides alter predator–prey interactions. *Biological Reviews* 82, 2007–2019.

Pyle, G. & Ford, A. T. (2017). Behaviour revised: contaminant effects on aquatic animal behaviour. *Aquatic Toxiology* 182, 226–228.

Qualls, C. W. (1989). Synergistic effect of imidacloprid and two entomopathogenic fungi on the behaviour and survival of larvae of *Diptera abbreviatus* (Coleoptera: Curculionidae) in soil. *Journal of Economic Entomology* 91, 110–122.

Rains, A. B. & Bajic, V. B. (2016). In silo toxicology: computational methods for the prediction of a chemical toxicity. *Wiley Interdisciplinary Reviews: Computational Molecular Science* 6, 147–172.

Rale, D., Reader, S. M., Sol, D., McDougall, P. T. & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews* 82, 291–318.

Rale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V. & Montgilio, P. O. (2010). Personality and the emergence of the pace-of-life syndrome at the population level. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365, 4051–4063.

Rearick, D. C., Ward, J., Venturrelli, P. & Schoenfuss, H. (2018). Environmental oestrogens cause predation-induced population decline in a freshwater fish. *Royal Society Open Science* 5, 180652.

Relyea, R. A. (2003). Prey cues and pesticides: a double dose of danger for amphibians. *Ecological Applications* 13, 1513–1521.

Rendón, M., Amouroux, D., Albert, C., Bérau, S., Bréthén, V. S., Gavriello, M., Grémillet, D., Hellman, H. H., Jakubas, D., Monbech, A., Striem, H., Tissero, E., Woyczulanis-Jakubas, K., Bentamante, P., et al. (2020). Contrasting spatial and seasonal trends of methylmercury exposure pathways of Arctic seabirds: combination of large-scale tracking and stable isotopic approaches. *Environmental Science & Technology* 54, 13619–13629.

Renick, V. C., Weinersmith, K., Vidali-Dorsch, D. E. & Anderson, T. W. (2018). Effects of a pesticide and a parasite on neurological, endocrine, and behavioral responses of an estuarine fish. *Aquatic Toxiology* 170, 335–343.

Rodriguez, A., Zhang, H., Klimander, J., Bronid, T., Anderson, P. L. & Anderson, M. (2016). ToxTea: a fast and robust software for tracking organisms. *Methods in Ecology and Evolution* 7, 459–464.

Rohr, J. R., Eklund, L., Smith, J. E., Stowers, J. R., Noldus, L. P. J. J., Bertram, M. G., DeBly, S., Wiles, S. C., Brendonck, L., & Pinckel, T. (2019). Populatiopn-, sex- and individual level range estimation and variance decomposition by generalized linear mixed-effects models. *Behavioral Ecology and Sociobiology* 73, 731–744.

Rohr, J. R., Kerby, J. L. & Shi, A. (2017). What’s your move? Movement as a link between personality and spatial dynamics in animal populations. *Ecology Letters* 20, 109–118.

Rohr, J. R., Kerby, J. L. & Shi, A. (2017). What’s your move? Movement as a link between personality and spatial dynamics in animal populations. *Ecology Letters* 20, 109–118.

Schiebel, C., Macri, S. & Porfiri, M. (2013). Acute ethanol administration affects zebrafish preference for a biologically inspired robot. *Alcohol* 47, 391–398.

Spiegel, O., Liu, S. T., Bell, C. M. & Shi, A. (2017). What’s your move? Movement as a link between personality and spatial dynamics in animal populations. *Ecology Letters* 20, 109–118.

Srinivasan, A. H. & Sokal, R. R. (1978). Linear and nonlinear mixed effects models. R Package version 3.1–131.

Symonds, M. R. E. & Moussalli, A. (2011). A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike’s information criterion. *Behavioral Ecology and Sociobiology* 65, 19–31.

Thapa, S., Kolev, N., Martin, J. M., Bertram, M. G., Wiles, S. C., Palacios, M. M., Bywater, C. L., White, C. R. & Wong, B. B. M. (2020). Chronic exposure to a pervasive pharmaceutical pollutant erodes among-individual phenotypic variation in a fish. *Environmental Pollution* 263, 114450.

Taylor, P., Crewe, T., Mackenzie, S., Lepage, D., Aubry, C., Crisler, Z., Finney, G., Francis, C., Goglieiro, C., Hamilton, D. & Holberton, R. (2017). The Motus wildlife tracking system: a collaborative research network to enhance the understanding of wildlife movement. *Aves Conservation and Ecology* 12, 8.

Thompson, J. A., Börger, L., Christiansen, M. J. A., Esteban, N., Laloe, J. O. & Hays, G. C. (2017). Implications of location accuracy and data volume for home range estimation and multimodel inference by generalized linear mixed-effects models. *Methods in Ecology and Evolution* 8, 1639–1644.

Thoré, E. S. J., Grégoire, A. F., Adriaenssens, B., Philippe, C., Stoks, R., Brendonck, L. & Pinckel, T. (2019). Population-, sex- and individual level range estimation and variance decomposition by generalized linear mixed-effects models. *Behavioral Ecology and Sociobiology* 73, 731–744.
Thöré, E. S. J., Brendonck, L. & Pinceel, T. (2021a). Natural daily patterns in fish behaviour may confound results of ecotoxicological testing. Environmental Pollution 276, 116738.

Thöré, E. S. J., Brendonck, L. & Pinceel, T. (2021b). Neurochemical exposure disrupts sex-specific trade-offs between body length and behaviour in a freshwater crustacean. Aquatic Toxicology 237, 105877.

Thöré, E. S. J., Van Hoorenwege, F., Philippe, C., Brendonck, L. & Pinceel, T. (2021). Generation-specific and interactive effects of pesticide and anionic surfactant exposure in a fish model call for multisstroser and multigenerational testing. Aquatic Toxicology 232, 105743.

Tickner, D., Oppermann, J. J., Abelli, R., Acreman, M., Arthington, A. H., Bunn, S. E., Cooke, S. J., Dalton, J., Darwall, W., Edwards, G., Harrison, I., Hughes, K., Jones, T., Leclère, D., Lynch, A. J., et al. (2020). Bending the curve of global freshwater biodiversity loss: an emergency recovery plan. BioScience 70, 330–342.

*TOLEDO, S., KISNON, O., ORCHAN, Y., SHOHAT, A. & NATHAN, R. (2016). Lessons and experiences from the design, implementation, and deployment of a wildlife tracking system. 2016 IEEE International Conference on Software, Technology, Architecture and Human Sideways Interaction (SWAT). Accessed 25.02.2021.

*TOLEDO, S., SHOHAMI, D., SCHIFFNER, I., LORIÈRE, O., ORCHAN, Y., BARTAN, Y. & NATHAN, R. (2020). Cognitive map-based navigation in wild bats revealed by a new high-throughput tracking system. Science 369, 188–193.

TOX21 (2020). Toxicology in the 21st Century (Tox21). Electronic file available at https://www.epa.gov/chemical-research/toxicology-testing-21st-century-tox21. Accessed 25.02.2021.

TOXCAST (2020). Toxicity Forcaster (ToxCast). Electronic file available at https://www.epa.gov/chemical-research/toxicity-forecasting. Accessed 25.02.2021.

TOXPi (2021). ToxPi: Toxicological Prioritization Index. https://oxpi.org/. Accessed 17.02.2021.

Tunbak, H., Vazquez-Prada, M. C., Ryan, T. M., Kämpe, A. R. & Drzeziot, E. (2020). Whole-brain mapping of socially isolated zebrafish reveals that lonely fish are not loners. eLife 9, e53863.

Tüzen, N., Müller, S., Koch, K. & Stoks, R. (2017).Pesticide-induced changes in personality depend on the urbanization level. Animal Behaviour 134, 45–55.

UNEP (United Nations Environment Programme) (2018). Review of laboratory and field testing of effects of pesticides on non-target species. Final report. UNEP, Nairobi, Kenya. Published by John Wiley & Sons Ltd on behalf of Cambridge Philosophical Society.

UNEP (2020). Global chemicals analysis of national and regional chemical inventories. www.epa.gov/chemical-research/toxicity-forecasting. Accessed 25.02.2021.

UNEP, UNEP-DESA, UN-Habitat (2018). Global land reserve: a tool for the analysis of national and regional national chemical inventories. Environmental Science & Technology 52, 2578–2584.

Wang, Z., Walker, G. W., Muir, D. C. G. & Nagatani-Yoshida, K. (2020). Toward a global understanding of chemical pollution: a first comprehensive analysis of national and regional chemical inventories. Environmental Science & Technology 54, 2578–2584.

Walker, V. R. (2004). Tuex, a fast multi-animal tracking system with markerless identification and 2D estimation of posture and visual fields. Animal Biotelemetry 7, 1–40.

Wang, Z., Walker, G. W., Muir, D. C. G. & Nagatani-Yoshida, K. (2020). Toward a global understanding of chemical pollution: a first comprehensive analysis of national and regional chemical inventories. Environmental Science & Technology 54, 2578–2584.

Westneat, D. F. (2011). Pesticides and fish behavior: a review of laboratory and field approaches. Current Opinion in Environmental Science & Health 5, 9–20.

Westneat, D. F., Wright, J. & Dinglemane, N. J. (2013). The biology hidden in residual waterbodies: new insights from neutral genetic variation. Biological Reviews 88, 729–743.

Westneat, D. F., Araya-Ajoy, Y. G., Allegue, H., Class, B., Dinglemane, N., Dochterman, N. A., Garamszegi, L. Z., Martin, J. G. A., Nakagawa, S., Réale, D. & Schielzeth, H. (2020). Collision between biological process and statistical analysis revealed by mean centring. Journal of Animal Ecology 89, 2013–2024.

Willett, M. & Klemey, A. P. (2019). An overview of behavioral, physiological, and environmental sensors used in animal biodemetry and biologging studies. Animal Biodemetry 7, 26.

Winter, M. J., Windell, D., Dytham, P., Pinion, J., Brown, J. T., Hetheridge, M. J., Ball, J. S., Owen, S. F., Redfern, W. S., Moger, J. & Randall, A. D. & Tyler, C. R. (2017). Dimensional functional profiling in the conserved trait-larval zebrafish brain. Scientific Reports 7, 6381.

Winter, M. J., Pinion, J., Tocchin, A., Takeono, A., Ball, J. S., Goodfellow, M., Metz, J., Treichel, M., Tai, X., Redfern, W. S., Hetheridge, M. J., Goodfellow, M., Randall, A. D. & Tyler, C. R. (2021). Functional brain imaging in larval zebrafish for characterising the effects of environmental pollutants acting via a range of pharmacological mechanisms. British Journal of Pharmacology 178, 2671–2699.

Wong, B. B. M. & Candolin, U. (2015). Behavioral responses to changing environments. Behavioral Ecology 26, 665–673.

Worley, T. A., Whalley, P., Hallsell, C., Rooney, A. A. & Walker, V. R. (2019). Systematic evidence maps as a novel tool to support evidence-based decision-making in chemicals policy and risk management. Environment International 130, 104871.

Yamakawa, O. & Takeuchi, R. (2018). UMATracker: an intuitive image-tracking platform. Journal of Experimental Biology 221, jeb182469.

Zimmerman, P. H. (2004). Systematic evidence maps as a novel tool to support evidence-based decision-making in chemicals policy and risk management. Environment International 130, 104871.

Zeure, A., Ieno, E. N., Walker, N., Savelyev, A. A. & Smith, G. M. (2009). Mixed Effects Models and Extensions in Ecology with R. Springer, New York, NY.

Zeure, A. F., Ieno, E. N. & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1, 3–14.

IX. Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Software available to be incorporated into behavioural ecotoxicology research in the laboratory.

Table S2. Hardware available to be incorporated into behavioural ecotoxicology research in the laboratory.

Table S3. Software available to be incorporated into behavioural ecotoxicology research in the field.

Table S4. Hardware available to be incorporated into behavioural ecotoxicology research in the field.