*Title Page

Contaminant-induced behavioural changes in amphibians: a meta-analysis

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Running title: How contaminants influence amphibian behaviour

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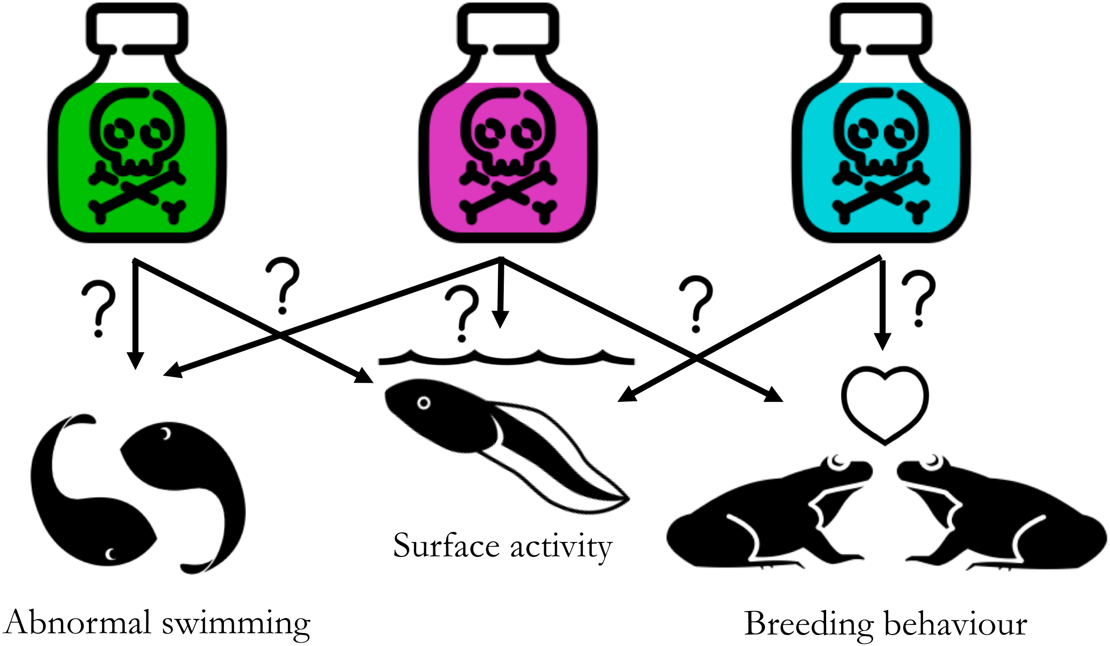
*Graphical Abstract



***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

Highlights

- Contamination is driving amphibian declines, but exposures are often sub-lethal
- Understanding how behaviour is altered helps determine ecological implications
- We used meta-analysis to quantify response types to a suite of contaminants
- Behavioural effects manifest in meaningful, predictable and repeatable ways
- This can help assess broader ecological impacts, if suitable data is provided

Abstract

Environmental contamination contributes to the threatened status of many amphibian populations. Many contaminants alter behaviour at levels commonly experienced in the environment, with negative ramifications for individual fitness, populations and communities. A comprehensive, quantitative evaluation of the behavioural sensitivity of amphibians is warranted to better understand the potential ecological impacts of contaminants. To this end, we conducted a systematic review and meta-analysis evaluating the magnitude and direction of behavioural changes following exposure to contaminants. Most studies were conducted in North America and Europe on larval stages, and most of these focused on the effects of insecticides. We found that a suite of contaminants influence a wide range of behaviours in amphibians, and that behavioural effects manifest in meaningful, predictable and repeatable ways, with insecticides typically invoking the strongest responses. In particular, insecticides increased rates of abnormal swimming, and reduced escape responses to simulated predator attacks. Our analysis identified five key needs for future research: (1) including greater details of experimental results, (2) developing a strong research base for future quantitative reviews, (3) broadening the suite of contaminants tested, (4) understanding effects of multiple stressors, and (5) establishing the ecological importance of behavioural alterations. Behavioural endpoints provide useful sub-lethal indicators of how contaminants influence amphibians, and coupled with standard ecotoxicological endpoints can provide valuable information for population models assessing the broader ecological consequences of environmental contamination. Continued efforts documenting behavioural responses to priority contaminants will enhance these capabilities.

Keywords

frogs; toads; tadpoles; ecotoxicology; behavior; HIREC

1. **Introduction**

Amphibians are one of the most imperilled taxa on the planet (Monastersky 2014) and are vulnerable to negative effects associated with a wide range of anthropogenic stressors (Alford and Richards 1999, Cushman 2006, Pounds et al. 2006). They may be particularly vulnerable to contaminant exposure since they often live and breed in areas designed to receive polluted waters (e.g. stormwater wetlands receiving urban runoff; Brand and Snodgrass 2010, Sievers et al. 2018c) and in locations where chemicals are intentionally added (e.g. agricultural wetlands receiving pesticides and fertilisers; Hazell et al. 2001). Many of the contaminants common in these environments are known to affect amphibian growth, development and survival, including heavy metals (Lefcort et al. 1998), pesticides (Egea- Serrano et al. 2012), hydrocarbons (Jelaso et al. 2002) and fertilisers (Marco et al. 1999). These impacts have been well conceptualised and discussed within the literature in several comprehensive reviews (e.g. Rohr and McCoy 2010, Egea‐Serrano et al. 2012, Baker et al. 2013). However, the ways in which common environmental contaminants influence amphibian behaviour have not been systematically assessed.

Many animals respond to environmental stressors by altering their behaviour (Tuomainen and Candolin 2011, Wong and Candolin 2015). These behavioural changes – intentional or otherwise – play a pivotal role in determining how well animals can be expected to cope in a changing environment (Saaristo et al. 2018). This is because many behaviours directly influence indicators of fitness such as survival, growth and reproduction, and because behavioural alterations tend to manifest in response to lower levels of environmental change than these fitness indicators (Zala and Penn 2004, Melvin and Wilson

2013, Montiglio and Royauté 2014). As such, behavioural responses are being increasingly used to assess the impacts of environmental stressors including contaminants.

Indeed, increasing evidence demonstrates that contaminants influence amphibian behaviour (Shuman-Goodier and Propper 2016) and researchers are measuring a myriad of behavioural endpoints to understand these effects. Some of the most common behavioural endpoints measured in larval amphibians include erratic swimming, surface swimming, feeding and overall activity levels, although a range of others have been used (Mitchkash et al. 2014, Moore et al. 2015, Miko et al. 2017, Sievers et al. 2018b). For example, widespread contaminants such as copper increase the time wood frog (*Lithobates sylveticus*) tadpoles spend swimming at the water surface (Hayden et al. 2015b), the herbicide glyphosate decreases activity and increases hiding in agile frog (*Rana dalmatina*) tadpoles (Miko et al. 2017), and the suite of contaminants in stormwater wetlands reduced the capacity of spotted marsh frog (*Limnodynastes tasmaniensis*) tadpoles to detect olfactory cues and ultimately interferes with normal anti-predator behaviour (Sievers et al. 2018d).

Given the dire status of global amphibian populations, an evaluation of the usefulness and sensitivity of behavioural responses for studying the ecological impacts of environmental contaminants in amphibians seems prudent. This will also help to identify avenues for future research that may lead to better understanding of how contaminants exert their sub-lethal effects on amphibians, and promote the inclusion of behavioural alterations as components of risk assessment and population modelling (O'Brien 2017). One of the challenges in synthesising behavioural research in toxicology is the lack of standardised methodologies that exists, making comparisons difficult (Melvin et al. 2017). As such, when assessing how contaminants affect behaviour, it is important that study designs accurately reflect natural conditions to correctly evaluate responses. The type of control – water or a solvent – for instance, might influence the direction or magnitude of impacts that are observed and hence the conclusions made about the broader ramifications of contaminants (Eddleston et al. 2012). Meta-analysis offers an opportunity to address these challenges by compiling data from many existing studies and quantitatively evaluating for important trends. Systematic evaluations of this sort can then provide the evidence to inform conservation decisions (Sutherland et al. 2004).

Here, we conduct a systematic review and meta-analysis to: (1) evaluate the sensitivity of behavioural endpoints by assessing the magnitude and direction of responses to various contaminants; (2) determine how the characteristics of experimental design may influence the outcomes of a study, and; (3) identify research gaps and highlight key recommendations. Since most animals are affected by multiple stressors simultaneously (Ormerod et al. 2010, Jackson et al. 2016), we also record studies that consider non-chemical stressors in addition to contaminants. Our intention here is not to delve into the nature and modes of action for different classes of contaminants, nor to discuss the physiological mechanisms causing behavioural changes. Instead, we focus on the utility of using behavioural responses to study contaminant effects in amphibians and conclude with considerations for future research.

2. **Methods**

2.1. Literature search

We performed a literature search on 6th December 2018 using ISI Web of Science (all databases) and the following term: (amphibia* OR frog OR toad OR newt OR salamander OR anuran OR caecilian) AND (behavio* OR locomotion OR attract* OR avoid* OR swim* OR perform* OR social interaction OR feeding OR forag* OR hiding OR hide OR navigat*) AND (contamin* OR toxic* OR pollut* OR light OR noise OR sound OR metal OR pesticide OR herbicide OR fertili* OR fungicide OR chemical OR pharma* OR petro* OR hydrocarbon OR acid OR nitr* OR UV OR ultraviolet OR salinity OR conductivity OR salt OR salts OR ozone OR disruptor OR inhibitor OR endocrine OR drug* OR estrogen OR pharama* OR therapuetant*). Initial results were filtered to include the following research areas: zoology, ecology, behavioural sciences, environmental sciences, toxicology, physiology, biology, evolutionary biology, multidisciplinary sciences, marine freshwater science, developmental biology, acoustics, and reproductive biology, with no restriction on publication date. In addition, we examined the reference lists of selected studies, including related reviews and meta-analyses. Excluding duplicates, we were left with a pool of 4,628 potentially relevant studies. The PRISMA flow diagram (Moher et al. 2009) shows the procedure used for selection of studies for the systematic review (Figure 1, and see Appendix 1 for a bibliography of the included studies).

2.2. Data extraction and classification

To be included, a study must have published original quantitative data on amphibian behavioural responses following exposure to a contaminant. We did not include field studies that compared exposed populations to unexposed populations (e.g. stormwater wetlands versus natural), since the contaminant(s) responsible for an observed behavioural response could not be conclusively identified and unknown confounding factors could not be ruled out. From each of the 123 studies we included, we recorded the location (continent and country), year of study, control type (water or solvent), contaminant class (e.g. insecticides, herbicides, fertilisers, metals, pharmaceuticals, salts), contaminant name (e.g. cadmium, atrazine), exposure concentration, duration of the experiment, additional stressors and stressor type (e.g. predator, warming), biological information of the test species (order, genus, species and life-history stage), number of replicates, and the behavioural response (endpoint) that was quantified (see Supplementary Table 1 for types of behavioural endpoints used in studies).

2.3.Effect-size calculation and statistical analysis

We extracted data from exposed and unexposed (control) groups from the text, tables or figures (using open source graphical digitiser software; Huwalt 2001) of the included studies and used this information to calculate response ratios (RRs): ln[RR] = ln[E] – ln[U], where ln[RR] is the log response ratio, E is the exposed group mean, U is the unexposed group mean (Hedges et al. 1999, 2008). For different behaviours, the direction of the RR can imply a positive or negative effect. For example, a positive RR for abnormal swimming would indicate higher rates of abnormal swimming in exposed individuals (a negative response to the contaminant), whereas a negative RR for feeding would be indicative of contaminant-induced reductions to feeding rate (another negative response).

For studies that considered the effect of different contaminants or multiple concentrations of the same contaminant, we calculated RRs for each. We also calculated RRs for each combination when two or more contaminants were investigated simultaneously. Our modelling approach (see below) ensured that studies with a high number of contaminants or concentrations were equally weighted.

A ln[RR] cannot be defined in situations where the numerator or denominator is zero, but adding a constant to these values can lead to serious bias (Rosenberg et al. 2013). As such, we took the more conservative approach of excluding these data from further analysis. However, to include as much of the available information as possible, if the response was provided as a percentage or proportion with one zero value (e.g. proportion individuals feeding; 0% versus 60%), we converted both values to represent the opposite (i.e. proportion not feeding; 100% versus 40%), and then reversed the sign of the resultant RR. This meant that only 62 RRs (2% of the dataset) were excluded from analysis.

Not all combinations of behavioural measures and contaminant classes were represented in the final dataset, precluding the exploration of complex interactions. Instead, for each behavioural measure we constructed separate generalised linear mixed-effects models with contaminant class (e.g. metal) or name (e.g. cadmium) fitted as fixed effects, and 'replicate' (contaminant-specific concentration) nested within 'study' (reference ID) fitted as a random effect (Mengersen et al. 2013a, Sievers et al. 2018a). Replicate nested within study accounted for any correlation amongst responses for a given contaminant and accounted for common contextual effects. The study random effect accounted for any systematic differences due to study-specific methodologies or biases. This model structure allowed us to analyse multiple RRs from within a given study rather than having to aggregate data to a single mean value per study, and to ultimately take into consideration the non-independence of multiple entries extracted from the same study (Krist 2011, Davidson et al. 2017).

When variance estimates are not provided in all studies within a meta-analysis, alternative weighting approaches are often used, such as weighting based on sample size (Mengersen et al. 2013b). Many of the studies included in our meta-analysis did not report sufficient information to calculate variance (e.g. no information or presented metrics such as inter-quartile ranges). This precluded the calculation of standard weightings used in some meta-analyses for all included studies (see Lajeunesse 2011, 2015). Instead of omitting a high proportion of studies or simply relying on unweighted analyses, we calculated weights based on the sum of sample sizes, reducing the influence of estimates based on less replicated studies (Stanley and Doucouliagos 2015). Furthermore, since the models used maximum likelihood methods, studies were automatically (implicitly) weighted by the uncertainty of the estimates, since the regression analyses and the variation in the regression estimates were included as part of the model (Mengersen et al. 2013a).

We produced unbiased parameter estimates and 95% confidence intervals using restricted maximum-likelihood estimation (REML) and suppressed intercepts. For each behavioural class, we also performed sub-group analysis for studies that included both water controls and solvent controls to assess differences in these experimental designs. We used the lmerTest package (Kuznetsova et al. 2015) in R v.3.2.2 (R Development Core Team 2015) to build models and extract least-squares means and confidence intervals (Stanley and Doucouliagos 2015).

3. **Results**

Most studies investigating contaminant-induced behavioural changes were conducted in North America (58%) and Europe (27%), with Australasia, Asia and South America contributing a relatively small proportion of the research effort (total 15%). After excluding papers with insufficient replication to quantitatively analyse behavioural responses (i.e. ventilation, proportion head out of water, oviposition-site selection) we were left with 112 studies and 2,553 RRs. Of these studies, the majority focussed on pesticides (64%) with fewer on metals (14%), fertilisers (13%), pharmaceuticals (8%), salinity (6%), and others (<5%) (note: summed percentages here and below are typically >100% as several studies focus on more than one contaminant, behaviour, developmental stage, etc.). Of the 72 studies on pesticides, 53% were on insecticides, 36% herbicides, 6% fungicides, and 5% others (e.g. molluscicides, surfactants). Metal studies focused on copper (31%), lead (25%), cadmium (19%), aluminium (13%), mercury (13%), arsenic (6%) and zinc (6%). Eight studies looked at pharmaceuticals, the majority of which were on endocrine disrupting chemicals (75%). In terms of developmental stages, most studies used larvae (81%), with only 16% studying adults and 6% metamorphs. In terms of behavioural endpoints, most studies measured activity levels (57%), followed by velocity (21%), feeding behaviours (21%), reproductive behaviours (11%), hiding (11%), abnormal swimming patterns (8%), escape responses (8%), and surface activity (6%). In addition, 7% of the studies measured rates of amphibian predation following exposure.

Exposure to contaminants increased rates of abnormal swimming (+168%), with most of the evidence coming from studies testing insecticides (Figure 2). Overall, activity levels were reduced in exposed individuals (-36%), again largely driven by responses to insecticides and, to a lesser extent, metals (Figure 2). Reproductive behaviours were reduced overall following exposure to contaminants (-20%), but confidence intervals were wide for individual contaminant classes even though all mean RRs were negative (Figure 2). Exposure to herbicides and insecticides reduced the capacity to exhibit escape responses (grand mean: -42%), but exposure to metals and fertilisers did not produce the same effect (although mean responses were still negative; Figure 2). Feeding rates were reduced in exposed individuals (-43%), with insecticides largely driving the trend of negative response (Figure 2). We found little evidence that exposure to contaminants affected levels of hiding, swimming speeds or surface behaviours, with confidence intervals overlapping zero (Figure 2). For forest plots of responses to each contaminant (e.g. atrazine, cadmium), see Supplementary Material.

Exposure to contaminants increased rates of amphibian predation – the culmination of multiple behaviours – when predators where not also exposed (+176%), but this trend reversed when predators were exposed along with the amphibians (i.e. the prey; -62%; Figure 3). We found little evidence that using a solvent control produced consistently different results to using a plain water control (Figure 4). Although RRs for hiding did differ statistically depending on the control type used, the size of this effect was small, and hiding was not a sensitive indicator of contamination. Out of the 112 studies, 42 considered an additional stressor, mostly the threat of predation (67%), acidity (12%) and temperature (warming; 10%).

4. **Discussion**

4.1. *Where and how the effects of contaminants on amphibian behaviour are being studied*

The majority of studies were conducted in North America, followed by Europe, mirroring the findings of other ecological meta-analyses (e.g. Conrad et al. 2011, Sievers et al. 2018a). Although not surprising, it is concerning since amphibian richness is highest in South America, Africa and Asia, and the proportion of threatened amphibians is greatest in the central and south American bioregion (Stuart 2008). The observed geographical bias towards affluent areas also raises issues surrounding the transferability of findings. For example, it is possible that the ecological impact of contaminants will be more severe in developing countries due to comparatively lower investment in, and less advanced technology for, pollution management (Kivaisi 2001). Conversely, these populations and species may be more adapted to polluted environments and exhibit reduced behavioural impacts. These uncertainties can only be addressed through increased research efforts in less studied regions, with comparison of the findings to existing research.

Most of research was conducted on larval amphibians (primarily tadpoles), likely due to a combination of (1) their fully aquatic lifestyle and fact that contaminants primarily enter aquatic environments, (2) larval life-stages being particularly vulnerable to predation, (3) easier ethics approval for studying tadpoles compared to adults, and (4) greater access to a larger number of comparable individuals relative to adult stages. Therefore, there is a dearth of information about the impacts of contaminants on adult amphibians, including effects of exposure during the larval phase that may not manifest until adulthood, such as changes in reproductive behaviour and/or success.

Decisions regarding experimental design can have a large impact on the conclusions that are drawn from a study (Skelly 2002). Nevertheless, in terms of control type, we found little evidence that the magnitude or direction of behavioural responses to contaminants differed when experiments used a solvent control or water control. Our findings are consistent with those observed for pesticides by Shuman-Goodier and Propper (2016), where the relative impact of exposure on swimming speed and activity was independent of control type. Since solvents can exhibit toxic effects or increase the toxicity of particular contaminants (e.g. Eddleston et al. 2012, Melvin et al. 2018), the choice of control used should be dictated by the study goal. For example, if the goal of the study is to quantify the impact of the active ingredient within a contaminant formulation, then using a solvent control (where the formulation uses one) is appropriate. For assessing potential ecological outcomes from contamination, we suggest that using a water control, and thus assessing the impact of the formulation against a contaminant-free environment, is more appropriate. However, when possible, experiments would ideally be conducted using both control and solvent-control; in this way, the effects of active ingredients and solvents can be partitioned to give a better idea of the casual factors of any behavioural changes.

4.2. How and which behaviours are being affected by contaminant exposure

We found that contaminants are broadly capable of influencing a wide range of behaviours in amphibians. Overall grand means (i.e. the mean behavioural response to all contaminants combined) were affected for many measures of behaviour, particularly for those that are well-studied (i.e. high replication). For these behaviours, contaminants typically had a reducing effect; that is, reducing rates of activity, breeding, feeding, normal swimming (i.e. opposite of abnormal swimming) and the ability of tadpoles to escape predation (Figure 2). In particular, our review highlights the considerable potential for insecticides to alter the behaviour of amphibians. Herbicides, fertilisers, endocrine disruptors and metals were tested as commonly as insecticides (i.e. often equal replication for response ratio calculations), but often had lesser impact.

Our findings match those from a meta-analysis on pesticides by Shuman-Goodier and Propper (2016), who found that pesticides reduced activity levels and swimming speeds in frogs and fish. Although our mean RR for speed matched that from Shuman-Goodier and Propper (2016), our confidence intervals slightly overlapped zero, potentially following the inclusion of more recent studies or as a result of our methodology (see Key Recommendations below). These authors found that changes in swimming speed were more variable than changes in activity levels and suggested that this may be due to the breadth of behavioural endpoints measured. For detailed discussion of the nature and modes of action for each contaminant-type and the physiological mechanisms causing changes to swimming speeds and activity levels, see Shuman-Goodier and Propper (2016).

Escape responses were hindered while rates of abnormal swimming increased following exposure to insecticides. Interestingly, Denoel et al. (2012) found that abnormal behaviours, such as swirling rapidly, only occurred in the presence of the pesticide, in this case the organochlorine endosulfan. On the other hand, Sievers et al. (2018b) examined abnormal swimming in uncontaminated water immediately following exposure to imidacloprid and still observed considerable increases in rates of erratic swimming and decreases in tadpoles' capacity to escape simulated predators. Such behavioural changes are likely to manifest into increased rates of predation in the wild.

Indeed, rates of amphibian predation increased following exposure to contaminants. However, this only occurred when predators were not themselves exposed. In studies that also exposed predators, predation rates declined substantially, often below rates when both predator and prey were unexposed (i.e. natural rates). The lack of effect following exposure to metals is driven by one individual study (Hayden et al. (2015a). For this study, we calculated response ratios for both non-lethal and lethal predatory attacks. These two measures, however, exhibited opposite trends, essentially cancelling each other out (a RR of zero); the frequency of non-lethal attacks was reduced, but lethal attacks increased, following exposure. The authors frame their conclusions as predation increasing under contamination, as the rate of fatal attacks (the more important and ecologically relevant measure) was much greater following exposure (Hayden et al. 2015a). Taken together, these findings suggest that under more natural conditions (i.e. when all animals are exposed to the same conditions), contamination may in fact lead to reduced predation upon amphibians, highlighting the need to assess the ecological effects of contaminants under realistic scenarios (also see Mandrillon and Saglio 2007).

4.3. Key recommendations

4.3.1. Including greater details of experimental results

We found several papers that did not provide quantitative data for behavioural responses with statistically non-significant differences between exposed and unexposed groups (i.e. when RRs would be close to zero, or when statistical power was low). Therefore, it is likely that mean response ratios would be closer to zero (i.e. no difference) for some behavioural responses if all possible data were available, but this is currently unknown. This is a well-known yet still pervasive issue extending beyond ecotoxicology and even beyond ecology (Csada et al. 1996, Koricheva 2003). In fact, the entire nature of statistical significance, with the assigned dichotomy of significant and non-significant has always been heavily criticised, and calls for its abandonment recur (Amrhein et al. 2019). Reiterating Parker et al. (2016), we strongly urge that all results (especially information needed to calculate effect sizes such as means, measures of variation, number of samples) are presented in future papers – at the least as supplementary information – regardless of statistical significance.

4.3.2. *Developing a strong research base for future quantitative reviews*

Since we included response ratios for all concentrations of contaminants tested (albeit with our nested random effect), the relatively large confidence intervals for specific classes of contaminants may reflect dose dependent responses. The nature of these dose-response relationships is not clear from our analysis (e.g. directional versus non-monotonic), and this is an avenue for future study. As more and more studies on contaminant-induced behavioural changes become available, future meta-analyses will have increasing confidence in the pooled evidence. With sufficient replication, these future quantitative reviews can also assess dose-response relationships that may exist, documenting contaminant-specific concentration thresholds below which no behavioural impacts are observed. Identifying whether the thresholds for observing behavioural responses occur at lower concentrations than other endpoints will be critical for future behavioural studies and goes to the heart of why behaviour should be studied more in ecotoxicological research. Ultimately, a strong research base will be invaluable for providing direction to regulators and industries that use or deal with these contaminants.

4.3.3. Broadening the suite of contaminants tested

Although studying prevalent and ubiquitous contaminants such as insecticides and herbicides is important, new and emerging contaminants desperately need greater focus, as impacts are largely unknown but potentially substantial. For example, endocrine disruptors (although these can include pesticides) have been highlighted as a probable cause of amphibian declines in the wild (Hayes et al. 2006, Orton and Tyler 2015), are well studied and have considerable impacts on fish (Jobling and Tyler 2003, Wedekind 2014). Endocrine disruptors are found in waste and surface waters around the world (Aris et al. 2014) and can impair the natural hormonal pathways of aquatic animals. However, the impacts of endocrine disruptors on amphibian behaviours are not well studied (although see Tamschick et al. 2016) despite evidence that these substances are highly relevant to amphibian populations (Lambert et al. 2015, Lambert and Skelly 2016, Bókony et al. 2018). Broadening the suite of contaminants being tested will help reveal how different contaminant classes with disparate modes of action influence behaviour, and will further help to unravel how amphibians might respond to exposure in the wild.

4.3.4. Exploring more the effects of multiple stressors

Our focus here was on investigating the effects of individual contaminants on amphibian behaviours. However, ecosystems are stressed in many ways, and it is important to identify how these additional stressors (e.g. presence of predators, warming) contribute to changes in behaviour in conjunction with chemical stressors. We found that 40% of studies investigated additional, non-chemical stressors. Although we deemed this insufficient to facilitate comprehensive analysis of the impact of additional stressors, this is a substantial body of research that represents a promising avenue for future quantitative analysis. Assessing interactions amongst multiple stressors can be extremely complex, but is critical to evaluate the impact of chemical and other threats under realistic conditions (Côté et al. 2016). This includes assessing behavioural responses to multiple stressors (Hale et al. 2017). Given that different interaction types can produce very different ecological outcomes, future research should investigate responses to combinations of stressors that occur in nature (Halfwerk and Slabbekoorn 2015). Understanding when and how stressors interact will be important for managing their effects, i.e. should management focus on mitigating one or more stressors, and if the latter, which?

4.3.5. *Establishing the ecological importance of alterations to behaviour*

Behaviours used to assess the impact of contaminants should have probable relevance for fitness outcomes such as responses to predator scents or simulated attacks. Some behavioural classes are more intuitively linked to direct fitness outcomes than others. For example, a reduced capacity to escape simulated predatory attacks (escape response) and higher rates of abnormal swimming would likely lead to increased susceptibility to predators, and thus, lower fitness. Similarly, reduced capacity or desire to feed or breed could culminate in reduced fitness and subsequent demographic effects. On the other hand, activity levels, surface activity, and swimming speeds and distance – particularly small changes – are less intuitively linked to individual fitness or demographic effects. Throughout the literature, links between such behavioural responses and fitness outcomes are often implied rather than directly tested, particularly in terms of predation rates (Bridges 1999, Sievers et al. 2018b). On occasion, opposite response types are interpreted in a way that suits the study narrative (typically, that the response is maladaptive). For instance, contaminant-induced reductions in overall activity – particularly when described as immobility or lethargy – have been suggested to increase predation due to a lower capacity to escape predators, whereas increased activity has been suggested to increase predation due to increased detectability (Azevedo-Ramos et al. 1992, Jung and Jagoe 1995, Lavorato et al. 2013, Sievers et al. 2018b).

Well-designed studies are necessary to link fitness outcomes to contaminant-induced behavioural responses and avoid misappropriation of responses. Hayden et al. (2015a) assessed the impact of copper on tadpole activity levels, and also on rates of fatal and nonfatal predatory attacks from dragonflies, highlighting how contamination can influence both predator and prey. From clever experiments like this, we can begin to tease apart the potential ecological implications of contaminant exposure. Ultimately, we suggest that future research on contaminant-induced behavioural responses in amphibians focuses on how behaviour correlates with fitness and how reduced fitness might scale-up to influence population demographics or even community-level metrics.

Interdisciplinary work between ecotoxicologists, behavioural ecologists and modellers can help to unravel these pathways (also see Relyea and Hoverman 2006). Population modelling provides tools to undertake targeted risk assessments that align with what needs to be protected, such as populations and communities (O'Brien 2017). Meta-analyses on behavioural and fitness responses to contaminants, particularly those that provide detailed information across all life-history stages, can provide a basis for understanding the population processes that mediate the effects of contaminants (O'Brien 2017). Models that use data on individual-level responses to predict demographic responses have long existed (Chapman 2002), but we have yet to see proper application of these tools, likely due to perceived costs, complexities, or lack of suitable data (see O'Brien 2017). We suggest that greater interdisciplinary collaboration will help break down these barriers, culminating in more effective predictions about the potential impacts of contaminants and how best to mitigate them.

5. **Conclusions**

Many contaminants influence a wide range of amphibian behaviours in biologically meaningful, predictable, and repeatable ways. Insecticides in particular strongly influenced amphibian behaviours, many of which intuitively suggest fitness reductions for exposed individuals. During our data extraction, we noted a pervasive issue that has long permeated ecology and ecotoxicology; many responses deemed ‘non-significant’ are not presented in publications, with likely implications for future studies and meta-analyses. Overall, our review suggests that researchers need to report all results of experiments regardless of statistical significance, continue to experiment with current and novel contaminants to build a strong research base, and expand experiments to try to unravel the true ecological implications of behavioural effects. Given that stressors rarely occur in isolation in nature, our understanding of contaminant impacts would also greatly benefit from more multiple stressor studies. We show that behavioural endpoints can provide useful indicators of contamination. The sub-lethal nature of behavioural responses also makes them a more ethical and perhaps more ecologically relevant endpoint to use in ecotoxicological research.

6. **Appendices**

Appendix A. The initial behavioural endpoints that data were extracted for, and the subsequent behavioural endpoints used in the meta-analysis.

Appendix B. Forest plots of weighted response ratios (and 95% CI on log scale) for all behaviours, split into contaminant class and name.

7. **Acknowledgements**

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8. Author contributions

MS, RH, KP, SS: Conceptualization; MS: Data curation; MS: Formal analysis; MS, RH, SS, KP: Investigation; All authors: Methodology; All authors: Visualization; MS: Writing - original draft; All authors: Writing - review & editing.

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Figure 1
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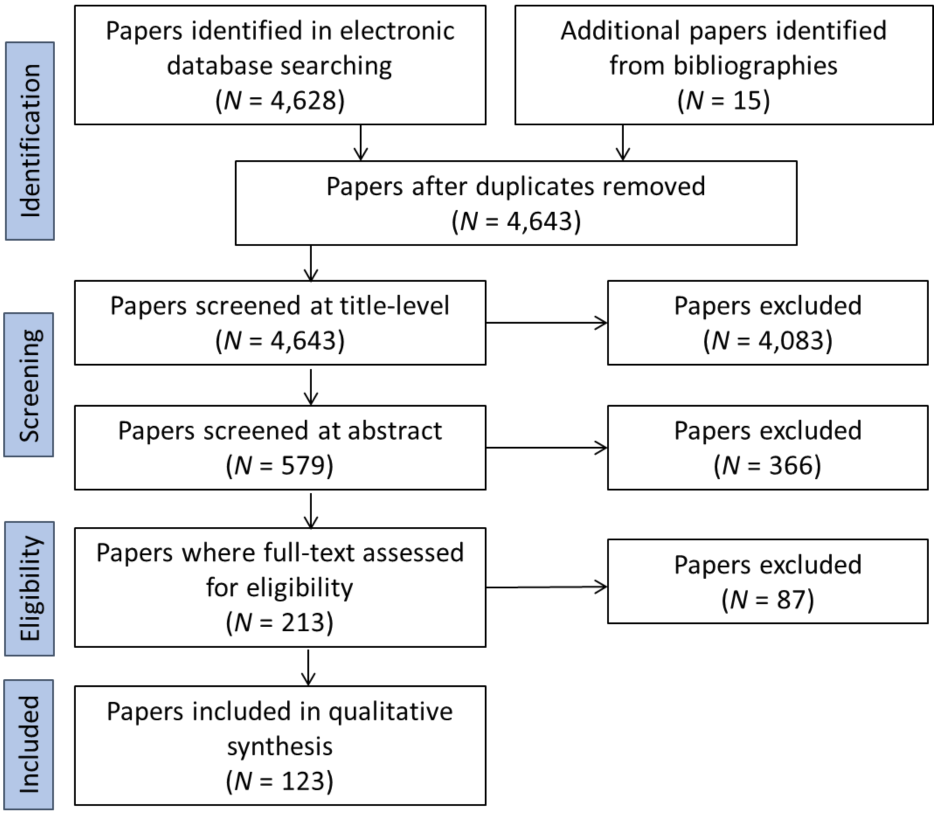

Figure 1. PRISMA flow diagram showing the procedure used for selection of studies for systematic review.

Figure 2

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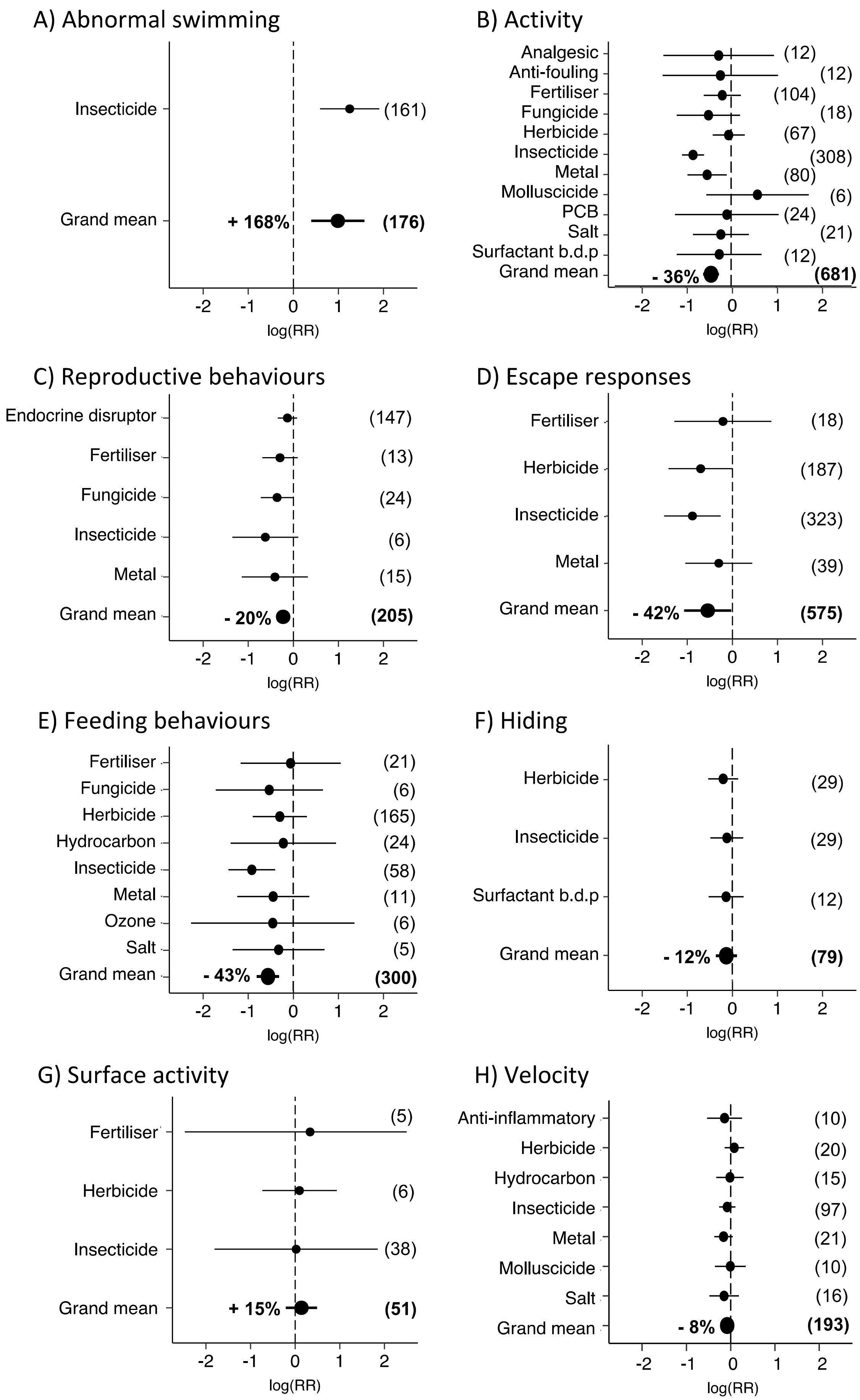

Figure 2. Forest plots of weighted response ratios (and 95% CI on log scale) for (A) abnormal swimming, (B) activity, (C) reproductive behaviours, (D) escape responses, (E) feeding behaviours, (F) hiding, (G) surface activity, and (H) velocity (where $n > 5$). We used weighted linear mixed-effects models with a unique identifier for each replicate and study fitted as a nested random effect (replicate within study), and intercepts were suppressed so that we could estimate separate coefficients. Grand means were estimated by removing contaminant-class as a factor from behavioural class-specific models. Numbers in brackets on the right indicate the number of response ratios within each data point, and grand means are boldface with percentage changes provided. PCB: poly; Surfactant b.d.p.: Surfactant break-down product.

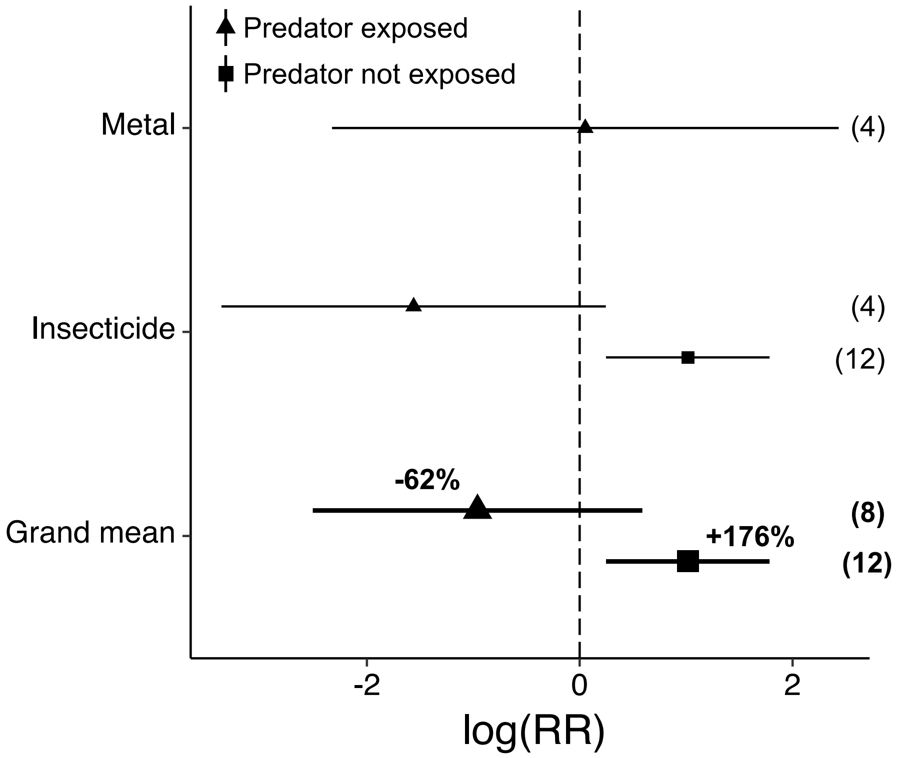

Figure 3. Forest plots of weighted response ratios (and 95% CI on log scale) for the rate at which amphibians were predated. We used weighted linear mixed-effects models with a unique identifier for each replicate and study fitted as a nested random effect (replicate within study), and intercepts were suppressed so that we could estimate separate coefficients. Grand means were estimated by removing contaminant-class as a factor from behavioural class-specific models. Numbers in brackets on the right indicate the number of response ratios within each data point, and grand means are boldface with percentage changes provided. For model details, see Figure 2 caption.

Figure 4
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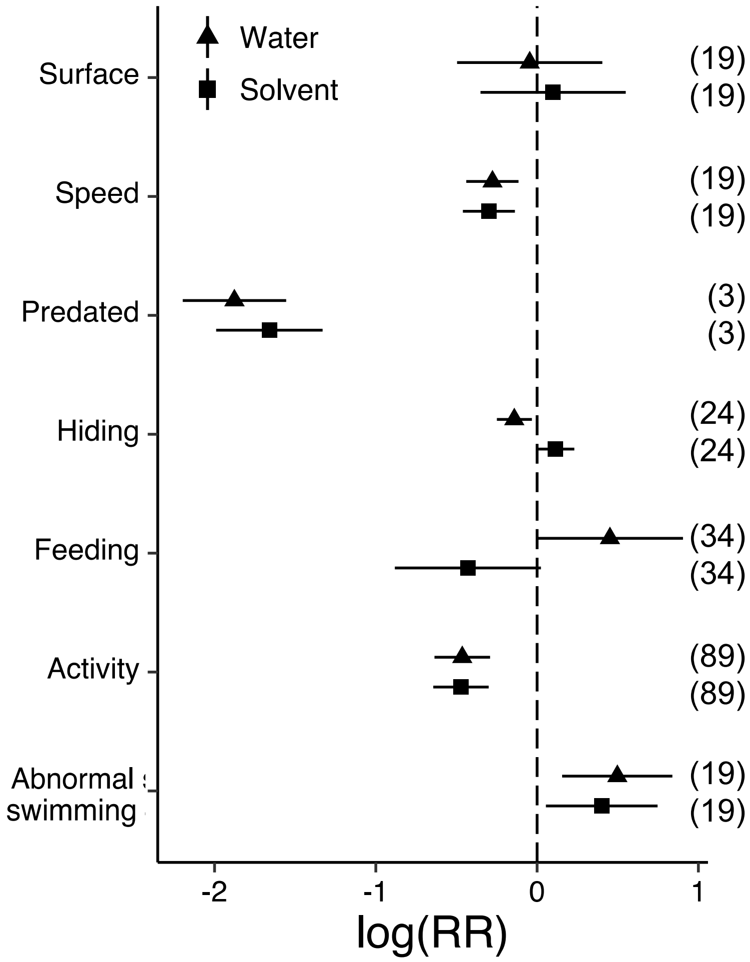

Figure 4. Forest plots of weighted response ratios (and 95% CI on log scale) of response to contaminants for seven behavioural endpoints comparing experiments with water (triangles) or solvent (squares) controls. We used weighted linear mixed-effects models with a unique identifier for each replicate and study fitted as a nested random effect (replicate within study), and intercepts were suppressed so that we could estimate separate coefficients. Numbers in brackets on the right indicate the number of response ratios within each data point.