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# A meta-analysis synthesizing the effects of pesticides on swim speed and activity of aquatic vertebrates

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# A meta-analysis synthesizing the effects of pesticides on swim speed and activity of aquatic vertebrates



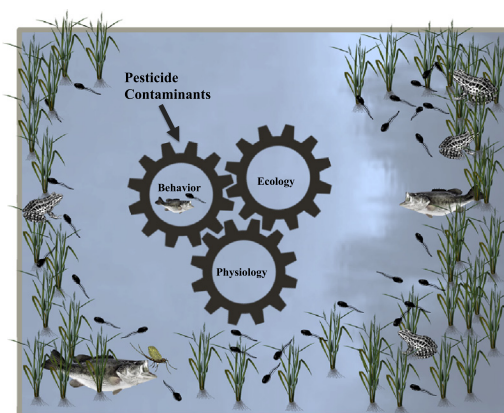
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## HIGHLIGHTS

- Pesticide contaminants are widespread and affect aquatic vertebrate behavior.
- We conducted a meta-analysis on the effects of pesticides on swim speed and activity.
- Environmentally relevant pesticides reduced swim speed and activity levels.
- Specific chemical classes may be targeted for further research and management.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Pesticide contaminants are ubiquitous in aquatic environments and pose a threat to biodiversity. Pesticides also have diverse mechanisms of action that make it difficult to identify impacts on exposed wildlife. Behavioral measures represent an important link between physiological and ecological processes, and are often used to generalize sub-lethal effects of pesticide exposure. In order to bridge the toxicological and behavioral literature, and identify chemical classes that denote the largest threat, we conducted a meta-analysis summarizing the effects of pesticides on swim speed and activity of aquatic vertebrates. We found that exposure to environmentally relevant concentrations of pesticides reduced the swim speed of exposed amphibians and fish by 35%, and reduced overall activity by 72%. There were also differences in the magnitude of this effect across chemical classes, which likely reflect underlying physiological processes. Pyrethroids, carbamates, and organophosphates all produced a large decrease in swim speed, whereas phosphonoglycines and triazines showed no overall effect. Pyrethroids, carbamates, organophosphates, organochlorines, and organotin also produced a large decrease in activity, while phosphonoglycines had no overall effect, and triazines had the opposite effect of increasing activity. Our results indicate that even sub-lethal concentrations of pesticides have a strong effect on critical behaviors of aquatic vertebrates, which can affect fitness and alter species interactions. We expect our synthesis can be used to identify chemical classes producing the largest sub-lethal effects for further research and management.

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Pesticide products used in agriculture are ubiquitous within the modern environment, and can act at multiple levels of biological organization (Fenner et al., 2013; Köhler and Triebkorn, 2013). Many pesticides are endocrine disrupting chemicals (EDCs) that alter fundamental physiological processes and lower fitness of exposed wildlife (McKinlay et al., 2008; Mnif et al., 2011). At larger scales, pesticide contaminants affect species interactions, decrease local and regional biodiversity, and even impair ecosystem function (Beketov et al., 2013; Colborn et al., 1993; Köhler and Triebkorn, 2013; Schäfer et al., 2007). Behavioral indicators, commonly used in ecological and toxicological studies to evaluate the effects of pesticides on aquatic organisms, create important connections between physiological and ecological processes (Clotfelter et al., 2004; Söffker and Tyler, 2012; Zala and Penn, 2004).

Aquatic ecosystems experience direct inputs of pesticide products from agricultural spray drift and run-off, and fish and amphibians represent valuable vertebrate indicators within these systems (Colborn and Thayer, 2000). Both taxa are also experiencing global declines, with approximately 34% of amphibian species and 39% of freshwater fish species currently threatened with extinction (Jelks et al., 2011; Wake and Vredenburg, 2008). Syntheses on the effects of environmentally relevant concentrations of pesticides on aquatic wildlife can provide direction for managers, reveal gaps and targets for future research, and encourage sustainable pesticide use in agriculture. Quantitative meta-analyses of the literature are particularly powerful given the range of pesticide products present in the environment, their variable mechanisms of action, and conceivable scope of influence.

Recent meta-analyses have summarized the lethal and sub-lethal effects of pesticides on amphibians, with varying results (Baker et al., 2013; Bancroft et al., 2008; Egea-Serrano et al., 2012; Ghose et al., 2014; Rohr and McCoy, 2010). Exposure to environmentally relevant concentrations of pesticides consistently reduces survival, with phosphonoglycines, carbamates, and chlorpyrifos producing the largest effect across studies (Baker et al., 2013). Sub-lethal effects have also been observed for the common metrics of gonadal morphology, sex hormone concentrations, immune function (Rohr and McCoy, 2010) and growth (Baker et al., 2013; Ghose et al., 2014). In contrast, for the measures of time to metamorphosis, hatching, and frequency of abnormalities, results range from no average effect (Egea-Serrano et al., 2012), to strong adverse effects (Boone and James, 2003; Jones et al., 2010; Smalling et al., 2015). Several reviews have also summarized sub-lethal effects of pesticides on fish for metrics including inhibition of acetylcholinesterase, disruption of steroid hormone production, alteration of gill and liver morphology, and induction of oxidative stress (Deb and Das, 2013; Gibbons et al., 2015; Slaninová et al., 2009).

Despite our understanding that pesticides also modify behavior of aquatic vertebrates at sub-lethal concentrations (Zala and Penn, 2004; Scott and Sloman, 2004), trends in behavioral responses have yet to be summarized by quantitative analyses. Behavioral measures are invaluable indicators of sub-lethal toxicity because they are highly sensitive, affordable, non-invasive, and represent an essential link between physiological and ecological processes (Kane et al., 2005; Melvin and Wilson, 2013; Scott and Sloman, 2004). Frequently affected behaviors include feeding, swim speed, and courtship that directly relate to survival and fitness in the natural environment (Baatrup and Junge, 2001; Logan and Matthiessen, 1984; Park et al., 2001). We argue the importance of summarizing the effects of pesticide exposure on behavior of aquatic vertebrates, given the evidence for strong indirect consequences on populations and aquatic community dynamics (Fleeger et al., 2003; Relyea, 2009; Rohr et al., 2006).

In order to synthesize experimental studies that bridge the toxicological and ecological literature, we conducted a meta-analysis on the effects of pesticides on two well established behavioral metrics: swim speed and activity level (Little and Finger, 1990). We targeted swim speed and activity levels because both are essential behaviors for

aquatic vertebrates, are easy to measure, and have been used consistently in experimental studies since the 1990's (Calfee et al., 2016; Little and Finger, 1990). The goals of our meta-analysis were three-fold: 1) to test whether exposure to environmentally relevant concentrations of pesticides produce generalizable effects on swim speed and activity 2) to evaluate the magnitude and direction of behavioral changes observed across common pesticide classes 3) to determine the relative importance of exposure time, concentration, choice of control, and publication biases on observed effects. We expect our synthesis can be used to identify chemical classes producing the largest sub-lethal effects for management and further research.

## 2. Methods

### 2.1. Selection criteria

We used the databases Science Direct and PubMed to identify primary literature using combinations of response variable keywords, "behavior\*", "predator response", "activity", and "courtship" with independent variable keywords; "pesticide\*", "amphibian\*", and "fish\*". A total of 3000 titles obtained from these searches were screened for initial relevancy, and 1238 studies were screened by abstract. We read the content of 167 articles, which included an additional 36 studies retrieved from the bibliographies of prominent reviews (Scott and Sloman, 2004; Zala and Penn, 2004; Bancroft et al., 2008; Egea-Serrano et al., 2012; Weis and Candelmo, 2012).

All studies that were included in the final analysis were required to meet the following criteria: 1) Studies were from the primary literature, and included an experimental manipulation of pesticide concentrations, where pesticide referred to any chemical herbicide, insecticide, fungicide, rodenticide or molluscicide. 2) Studies used a valid control, defined as either water or solvent vehicle containing no pesticide product. 3) Means, sample sizes, and variance (standard error, standard deviation, or confidence intervals) were included for at least one of the behavioral endpoints swim speed or activity level. 4) Studies used ecologically relevant concentrations of pesticides, representative of concentrations wildlife could encounter in natural aquatic habitats. If authors did not explicitly state that concentrations were ecologically relevant, we based decisions on values summarized in a prior meta-analysis (Baker et al., 2013), or on the highest plausible concentrations that have been detected within aquatic environments (SI Table 1.2). We included concentrations observed in rice paddies, as these systems can harbor both amphibians and fish (Anyusheva et al., 2012; Machado and Maltchik, 2010). If pesticide treatments exceeded these upper limits, they were not included for analysis. 5) Studies stated the number of hours or days that the animals were exposed to a pesticide. If studies measured behavior throughout life history stages, or throughout varying lengths of time, the final time point was considered (for example, Rohr and Palmer, 2005). Similarly, if studies exposed animals for varying lengths of time, the longer exposure was included for analysis. 6) If papers looked at the effects of pesticides in combination with other treatments, such as response to predators or stimuli, only interaction free treatments were included. In the case of one study, which manipulated temperature within the natural range of the organism, one temperature treatment was chosen at random to include for analysis (Broomhall, 2006).

The majority of studies that met all of our selection criteria contained multiple comparisons among species, concentrations, and pesticide types. When this was the case, all comparisons were included for analysis. Within study comparisons were treated as independent samples if they had a separate corresponding control. For example, Little et al. (1990) evaluated six different pesticides, each with a corresponding control, whereas Yen et al. (2011) evaluated four different pesticides using one control. If the same control was used for multiple comparisons, two separate models were tested to determine if non-independence was altering the grand mean effect size for activity and swim

speed, respectively (see model selection). Where non-independence was shown to have no significant effect on grand effect sizes, all concentrations of ecological relevance were included for analysis.

## 2.2. Effect sizes

We used the log response ratio,  $LnR = Ln(\frac{X_{\sim 1}}{X_{\sim 2}}) = Ln(X_{\sim 1}) - Ln(X_{\sim 2})$ , as our effect size measurement, where  $X_{\sim 1}$  was the treatment mean and  $X_{\sim 2}$  was the control mean. The log response ratio is a robust choice for measuring effect size as it can be easily converted into a measure of percent change ( $\% \Delta = \exp((LnR) - 1) * 100$ ), is less sensitive to differences between units, and normalizes data that is not normally distributed (Lajeunesse, 2011). We used the R package Metafor to compute the log response ratio and the associated pooled variance,  $\sigma^2(LnR) = \frac{SD_1^2}{N_1 X_{\sim 1}^2} + \frac{SD_2^2}{N_2 X_{\sim 2}^2}$ , for each control and treatment comparison (Lajeunesse, 2011; R Core Team, 2016; Viechtbauer, 2010). Negative effect sizes represented a reduction in swim speed or activity levels in response to pesticide exposure, whereas positive effect sizes represented an increase.

## 2.3. Model selection

Two separate random effects models were used to calculate mean effect size for the behavioral endpoints activity and swim speed. We used a random effects model because we expected large heterogeneity between our effects due to differences in experimental designs, measurements, and sampling methods. The random effects model assumes the studies we included for analysis are representative of the effect that would be observed within a greater population of studies, and thus can also be used to make more powerful inferences than a fixed effects model (Hedges and Vevea, 1998). In Metafor we obtained grand mean effect sizes and 95% confidence intervals for both activity and swim speed using the rma.uni function, and tested for significant heterogeneity of variance.

To evaluate the influence of non-independent comparisons we compared the grand mean effect sizes where data was 1) averaged to remove multiple comparisons or 2) where all comparisons were included for analysis. Where no effect on the grand mean was found, all comparisons were included in subsequent analysis. Finally, we used mixed effect models to test for influence and relationships between moderators on our grand effect sizes. We tested for the influence of chemical class, type of control (solvent or water), exposure duration, taxa, exposure concentration, and the unit of replication. These were variables we expected to influence behavioral response to pesticide exposure. Final models with best fitting predictors were chosen based upon Akaike Information Criterion (AIC) using a two-increment cut off and the restricted maximum likelihood method (REML). Throughout all model selection we used Metafor's default weighting function, which weights studies by the inverse variance so that those with larger variance contribute less to the overall effect size and those with small variance contribute more.

## 2.4. Publication bias

We used three approaches to test for publication bias. First, we plotted the residuals from our final mixed effects models against their standard error in contour enhanced funnel plots (Peters et al., 2008). Assuming that studies with smaller variance will be more precise and that studies with larger variance will be less precise, data without publication bias should resemble the shape of a funnel (Peters et al., 2008; Rothstein et al., 2006). Second, we tested for significant asymmetry in our funnel plots using regression tests in Metafor (Viechtbauer, 2010). Third, we calculated Rosenthal's fail safe number, which represents the number of papers that would need to be published with non-

significant results to change the mean effect sizes we calculated for each behavioral outcome to non-significant (Rothstein et al., 2006).

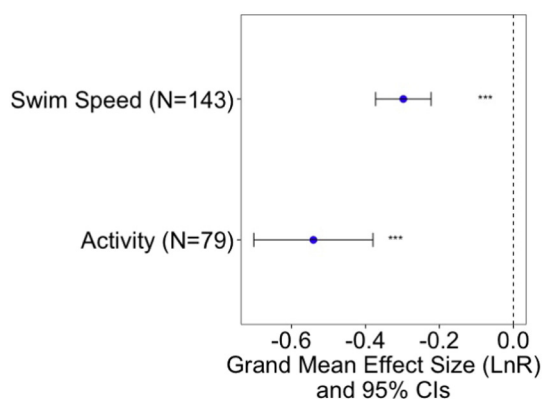
## 3. Results

A total of 39 papers from the published literature met our inclusion criteria. We examined behavioral measures separately, resulting in a total of 27 studies with 143 comparisons for swim speed, and a total of 17 studies with 78 comparisons for activity. For swim speed, a total of 34 of these comparisons were for amphibians and 109 were for fish, and for activity 56 comparisons were for amphibians and 22 were for fish. Seven chemical classes, representing 23 different pesticides, were represented.

### 3.1. Swim speed

The average effect of pesticide exposure on swim speed generated with our random effects model was  $-0.298$  (Fig. 1, Table 1,  $p < 0.0001$ ). This value translated to a 34.7% decrease in swim speed in response to pesticide exposure ( $\pm 3.80\%$  SE). We observed significant heterogeneity in our random effects model ( $p < 0.0001$ ), which indicated that additional variance between studies might be explained by moderators (Table 1). Using model selection, we determined that chemical class, length of exposure, concentration, taxa, the types of replicates and control used in the experiment were the moderators that best explained the variance in our mixed effects model. Together these moderators accounted for an additional 44.3% of the observed heterogeneity. Differences in the magnitude of the effect on swim speed were detected between different chemical classes and replicate types, and significant variation remained within the model (Table 1,  $p < 0.0001$ ).

We calculated mean effect sizes and 95% confidence intervals for group moderators, and found that swim speed was reduced by exposure to carbamates, organochlorines, organophosphates, and pyrethroids, but was not affected by phosphonoglycines or triazines (Fig. 2a, SI Table 1.1). Fish appeared to be more sensitive than amphibians, and studies that used individual replicates reported larger effects than those that used whole tank or pooled individual replicates (Fig. 2d and c). We also observed no significant difference in the type of control used; indicating that either water or solvent-based controls could represent an acceptable design (Fig. 2b). No relationship was observed between exposure duration or concentration and swim speed (SI Table 1.1).



**Fig. 1.** Grand mean effect sizes and 95% confidence intervals of the effect of pesticides on swim speed and activity.  $N$  represents the total number of comparisons used to calculate the mean. The total number of individual studies included in the analysis was 27 and 17 for swim speed and activity, respectively.

**Table 1**

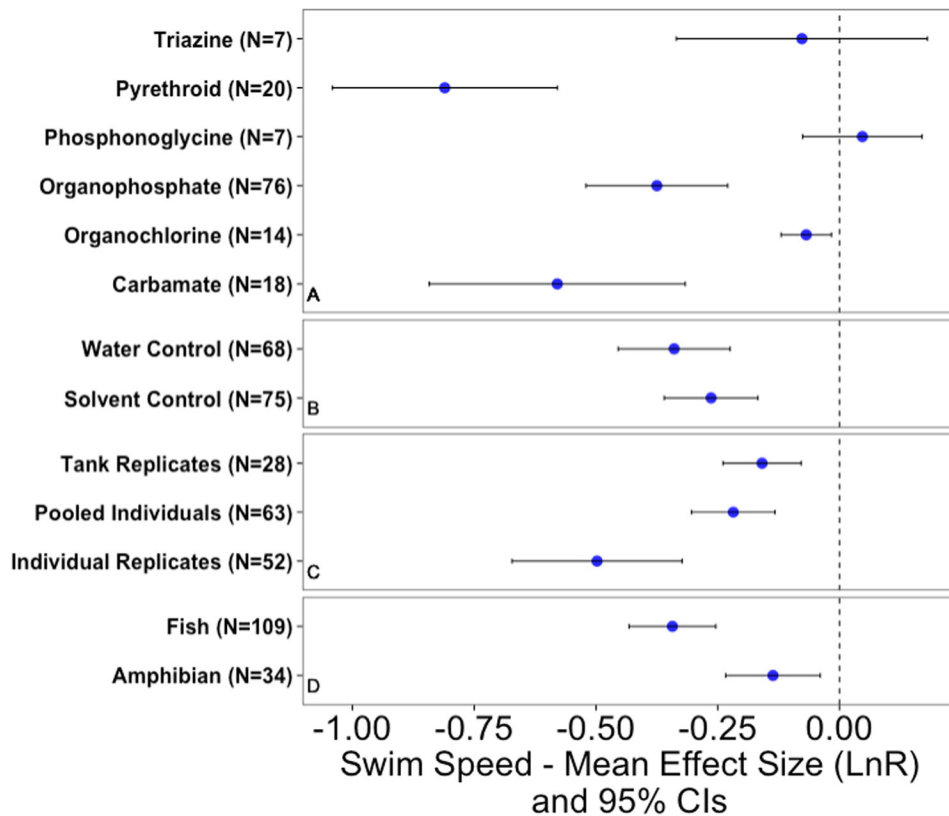
Random effects models and final mixed effects model outcomes for the behavioral metrics swim speed and activity, with heterogeneity Q statistics. For swim speed, the final mixed effects model included the moderators time, chemical class, concentration, replicate type, and taxa. For activity, the final mixed effects model included the moderators time, chemical class, replicate type, and control type.

95% confidence interval							
	Estimate	SE	Lwr	Upr	Q	df	p
Swim speed							
1. Random effect model	0.30	0.04	−0.37	−0.22			
Test of residual heterogeneity					5041.94	142	0.0001
2. Final mixed effects model ( $R^2 = 44.27\%$ )							
Test of residual heterogeneity					2744.39	144	0.0001
Test of moderators					95.91	11	0.0001
Activity							
1. Random effects model	0.54	0.08	0.70	0.38			
Test of residual heterogeneity					23,863.33	77	0.0001
2. Final mixed effects model ( $R^2 = 30.16\%$ )							
Test of residual heterogeneity					7592.04	65	0.0001
Test of moderators					41.23	12	0.0001

### 3.2. Activity

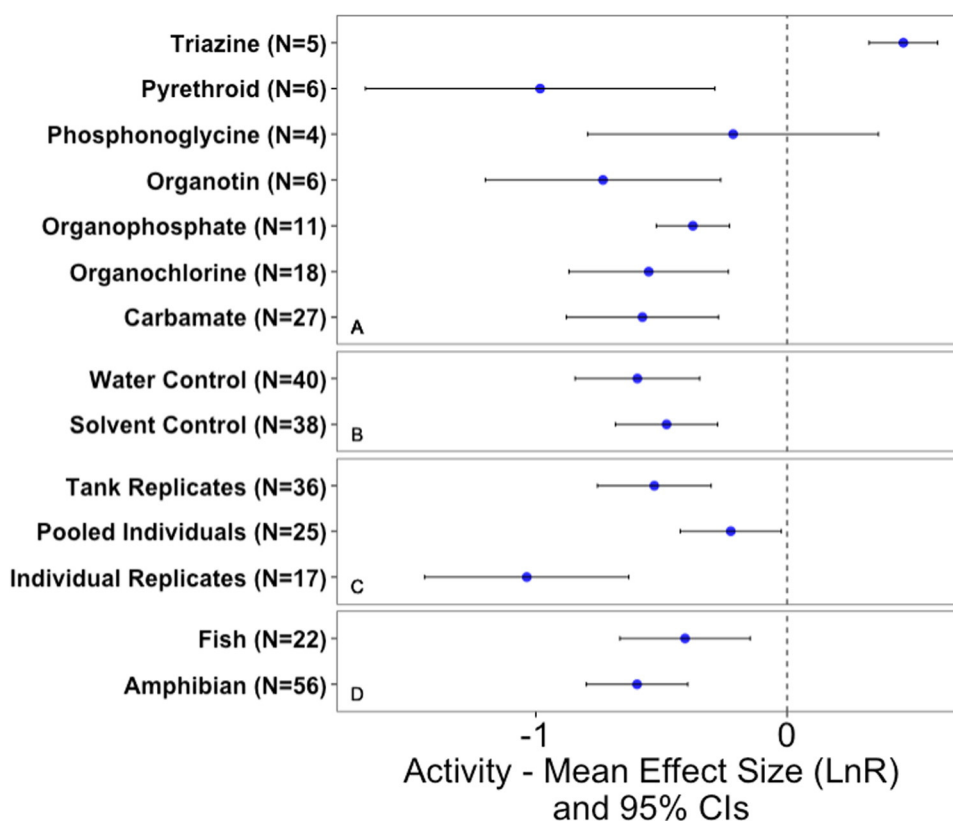
The average effect of pesticide exposure on activity generated with our random effects model was  $-0.541$  (Fig 1, Table 1,  $p < 0.0001$ ). This value translated to a 71.8% decrease in activity level in response to pesticide exposure ( $\pm 8.2\%$  SE). We observed significant heterogeneity in the model, which indicated that additional variance between studies might be explained by moderators (Table 1). Chemical class, length of exposure, replicate type, and the type of control used in the experiment best explained the variance in our model. Together these moderators accounted for an additional 30.2% of the heterogeneity in our mixed effects model (Table 1).

We also examined the mean effect sizes and 95% confidence intervals for grouped moderators (SI Table 1.1). Pyrethroids, organotins, organophosphates, organochlorines, and carbamates all produced a similar effect of reducing activity levels, whereas triazines increased activity levels relative to other classes, and phosphonoglycines had no effect (Fig. 3a). We observed no differences in the average effect size between amphibians and fish, or in the type of control used (Fig. 3d and b). We again found evidence that studies that used individual replicates for behavioral analyses produced a larger average effect size, a result that is comparable to those seen for swim speed. For the continuous variable time we found that an increase in exposure duration corresponded with a decrease in the observed negative effect on activity



**Fig. 2.** Mean effect size and parametric 95% confidence intervals of the effect of (A) chemical class, (B) type of control, (C) replicate type, and (D) taxa on swim speed. Effect sizes were considered to be significant if the confidence intervals did not overlap with zero, and differences between groups were significant if confidence intervals did not overlap with each other. N represents the total number of comparisons, and negative values represent a decrease in swim speed.





**Fig. 3.** Mean effect size and parametric 95% confidence intervals of (A) chemical class, (B) type of control used, (C) replicate type, and (D) taxa on activity. Effect sizes were considered to be significant if the confidence intervals did not overlap with zero, and differences between groups were considered significant if confidence intervals did not overlap with each other. N represents the total number of comparisons, and negative values represent a decrease in activity level.

level (SI Table 1.1). Finally, we found no relationship between exposure concentrations on activity in the studies included for this analysis (SI Table 1.1).

### 3.3. Publication bias

Using regression tests, we found that there was no significant asymmetry in our final mixed effects model funnel plots for swim speed ( $t = 1.351$ ,  $df = 129$ ,  $p = 0.18$ ) or for activity ( $t = 1.163$ ,  $df = 65$ ,  $p = 0.25$ ). Although results from this regression test should be interpreted with care, it does indicate a lack of publication bias within the literature for reported effects of pesticides on swim speed and activity (SI Figs. 1.1 and 1.2). Rosenberg's fail-safe number was also robust for both swim speed (48,569) and activity (1062), indicating that a large number of studies would need to be published with non-significant results to alter our conclusions.

## 4. Discussion

We found that environmentally relevant pesticide concentrations consistently reduced swim speed and activity levels of exposed fish and amphibians in experimental studies. The large magnitude of observed changes, a 35% reduction in swim speed and a 72% reduction in activity levels, suggest cascading consequences for exposed populations.

Pyrethroids, which had the largest average effect on both swim speed and activity, are among the most common insecticides used within agriculture (Alonso et al., 2012). Long-term monitoring of pyrethroids often reveals low detection frequencies in aquatic ecosystems, due to the hydrophobic properties of this class of pesticides and the pulsatile release from agricultural systems (Liu et al., 2004; Rasmussen et al., 2013b). However, studies that have conducted water and sediment

sampling during or after spraying in nearby agricultural fields have reported concentrations ranging from  $1.1\text{E-}5$  to  $0.038$  mg/L (Allinson et al., 2015; Johansson et al., 2006; Kuivila et al., 2012). This range is comparable to the findings of a recent meta-analysis, which determined that reported environmental concentrations of pyrethroid pesticides exceed regulatory threshold limits in the US and Europe over 65% of the time (Stehle and Schulz, 2015). Pyrethroid concentrations found in the environment commonly exceed the average concentration included in our analysis,  $4.92\text{E-}03$  mg/L, and overlap with the established lethal doses (LC50s) for both fish and amphibians (Johansson et al., 2006; Stehle and Schulz, 2015). Given that we also found that shorter exposure times, which may realistically reflect pulse exposure events in the environment, produced a larger effect on activity, we believe pyrethroids represent a chemical class to which benthic aquatic vertebrates may be particularly vulnerable.

Organophosphates and carbamates are two structurally similar insecticide classes that also produced a large decrease in both swim speed and activity level. Organophosphates can disrupt thyroid hormone function, cause immune and nervous system toxicity, and impair metabolic function of non-target organisms (Köhler and Triebkorn, 2013). The use of several organophosphate insecticides has been discontinued in the United States, and a preliminary risk assessment by the Environmental Protection Agency has summarized significant human and environmental health concerns for seven additional organophosphates (US EPA, 2015). Carbamate insecticides exhibit similar toxicity to non-target organisms (Baldwin et al., 2009; Jarrard et al., 2004; Köhler and Triebkorn, 2013; Moore and Teed, 2013). Representatives within both classes are still used in the United States and internationally, and behavior of fish and amphibians appears to be highly responsive to environmentally relevant exposures.

The organochlorine pesticides tested by studies in this analysis, endosulfan and chlordane, decreased swim speed and activity levels of

both fish and amphibians (Figs. 1 and 2). While chlorodane is no longer used, and endosulfan is currently being phased out in the US, by 2016, both compounds persist in aquatic environments, biomagnify, and bioaccumulate (Chopra et al., 2011; Weber et al., 2010; Zeiss, 2014). Organochlorine pesticides including DDT-like compounds are considered persistent organic pollutants, and usage is tightly restricted and regulated in the United States and Europe. However, products within this class are still widely used and detected in Southeast Asia (Ali et al., 2014; Syed et al., 2014; Yadav et al., 2015). Organochlorines can also travel long distances in the atmosphere, and remarkably high concentrations have been observed in otherwise isolated arctic wildlife and humans (Lana et al., 2014; Pedersen et al., 2015).

Triazines, in particular the herbicide atrazine, are globally used to control weeds in agricultural crops, and are often detected in aquatic ecosystems (Gfrerer et al., 2002; States, 2000; Van Dijk and Guicherit, 1999). Interestingly, atrazine was the only triazine tested by studies in our analysis, and it was also the only chemical to cause a significant increase in activity level of both fish and amphibians. These results are consistent with a recent analysis summarizing effects of atrazine on fish and amphibians, which found an increase in locomotor activity in 12 out of 13 studies examined (Rohr and McCoy, 2010). One likely mechanism driving this observed increase in locomotor activity may be disruption of the dopaminergic systems as has been observed in rats (Bardullas et al., 2011; Rodríguez et al., 2013). Observed increases in activity levels do not appear to cause a corresponding increase in swimming speed, indicating that the two behaviors are not necessarily correlated. Although significant debate surrounds whether atrazine poses a threat to aquatic systems (Boone et al., 2014; Rohr and McCoy, 2010; Solomon et al., 2008 and 1996), our results suggest that exposure can alter an important behavior of aquatic vertebrates.

Our findings that swim speed was more variable in response to different chemical classes than activity may reflect differences in the breadth of the behavioral endpoints we measured. Activity is a general behavior likely to be affected by multiple underlying mechanisms, whereas changes in spontaneous swim speed can be more explicitly linked to fewer, specific mechanisms, such as acetylcholinesterase mediated inhibition of neuroendocrine tissue (Khalil et al., 2013). If AChE inhibition is the principal mechanism responsible for reductions in swim speed, then pesticides that are not AChE inhibitors likely produce a smaller effect. Furthermore, there is clearly a need for behavioral analyses examining other common classes such as neonicotinoids, as well as increased breadth within classes. Not all chemical classes of pesticides are represented in our analysis, and within classes we had a relatively small representation of active ingredients. For example, we only had three active ingredients represented with the carbamate class, when there are thousands of products available for use containing over 50 active ingredients for that chemical class alone (Hill, 2003).

Pesticides that serve as endocrine disruptors often exhibit non-monotonic dose response curves. When this is the case, low concentrations can often produce larger or even opposing effects compared with high concentrations due to differences in receptor sensitivity, saturation levels, hormonal interactions, and life history (Brodeur et al., 2013; Harris, 2005). Several pesticides included in this analysis, such as atrazine, diazinon, and permethrin, produce distinctive non-linear responses in fish and other taxa (Jeffries et al., 2015; Rohr and McCoy, 2010; Timofeeva et al., 2008; Vandenberg et al., 2012). We believe this complexity is responsible for the high heterogeneity of the response of swim speed and activity across the concentrations we summarized, which ranged from 6.00E-07 to 15 mg/L. Furthermore, the diminishing effect of pesticide exposure on activity level over time, as we found in this study, is consistent with theories of sensory adaptation and sensory fatigue (Wark et al., 2007). Behavioral adaptation can occur when prolonged exposure to constant stimuli, such as sub-lethal pesticide exposure, alters the relationship of excitatory and suppressive signals at the level of the receptor (Solomon and Kohn, 2014). Overall, considering the large effects of short exposure times and low concentrations included in our analysis, our findings support other conclusions that behavioral endpoints are

highly responsive measures of pesticide exposure and sub-lethal toxicity, on average more sensitive than other physiological metrics (Söffker and Tyler, 2012; Melvin and Wilson, 2013).

Experiment design can also contribute to biases in effect size, and influence scientific conclusions (Cumming, 2014; Hurlbert, 2009). For instance, the use of a solvent control is encouraged when the compound being investigated is not readily soluble in water. In these cases, a water control may not be sufficient because solvents can increase the toxicity of some pesticides (Eddleston et al., 2012). However, in our analysis we found no differences in the effect size of studies that used solvent versus water controls, suggesting that at least for the pesticides included in this analysis the use of either may be appropriate. Nevertheless, we encourage researchers to use controls that most clearly match the experimental condition. We also found that the experimental units defined for behavioral analyses varied across studies, and were often difficult to discern within reported methodology. Studies that used individual replicates as the experimental unit showed a larger average effect than those using tank replicates. For both swim speed and activity we illustrated a large effect of replicate choice on statistical significance, even when using a measure of effect size, and recommend that the experimental unit of replication be considered carefully and reported with much greater transparency (Hurlbert, 2009). The experimental unit, particularly with plastic behavioral endpoints, should also be chosen to reflect biological relevancy. For example, if schooling fish are housed individually, a large observed effect on activity might be confounded by a response to an unrealistic treatment. Furthermore, experimental studies in the field are rare, and all of the studies we reported here were conducted in the laboratory. Future comparative field and laboratory research should be prioritized as there is evidence for diminished behavioral responses in complex environments (Mikó et al., 2015).

Changes in behavior, including swim speed and activity level, can be directly linked to survival, growth, and reproduction of aquatic vertebrates. For instance, flathead minnows exposed to the pyrethroid esfenvalerate experienced reduced activity levels, which resulted in higher predation and reduced growth rates (Floyd et al., 2008). Similarly, behavioral reductions in swim speed and activity in juvenile chinook salmon exposed to organophosphate and carbamate pesticides were linked to reduced growth, feeding, and size at migration (Baldwin et al., 2009). Sub-lethal effects of pesticides on species interactions, such as predation and competition, are also common in our modern environment and have the potential to shape the life history of a variety of taxa (Campero et al., 2007; Pestana et al., 2009; Janssens and Stoks, 2012; Rasmussen et al., 2013a). For example, Relyea and Edwards (2010) found that reduced activity levels in tadpoles as a result of sub-lethal pesticide exposure reduced predation rates by newt predators. Bridges (1997) reported a trade-off where tadpoles with reduced activity levels experienced lower predation rates by newts, but also decreased growth and delayed development. The direction of these interactive effects is not always intuitive, and reflects a pressing need to incorporate ecological complexity into toxicological studies (Chapman, 2002; Köhler and Triebkorn, 2013).

Behavioral changes also produce indirect effects on whole community structure by altering species interactions and triggering trophic cascades (Boone and Semlitsch, 2002; Fleeger et al., 2003; Rohr and Crumrine, 2005; Söffker and Tyler, 2012). Paying due attention to behavior can thus integrate concepts of community ecology to the field of toxicology to improve the way we evaluate pesticides (Rohr et al., 2006; Söffker and Tyler, 2012; Van den Brink et al., 2002). Future research should continue to use behavioral measures to examine direct and indirect effects of pesticide contaminants on fitness, populations, communities, and ecosystems.

## 5. Conclusions

We found that swim speed and activity levels were both highly responsive to environmentally relevant pesticide exposure. Swim speed

of exposed amphibians and fish was reduced by 35%, and overall activity was reduced by 72%. We suggest that researchers continue to investigate the consequences of sub-lethal pesticide exposure for wildlife by incorporating measures of behavior, thus linking effects on internal physiology to the external environment.

### Conflict of interest

There is no conflict of interest.

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### Appendix A. Supplementary information

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2016.04.205>.

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