Cover page

Title:

The impacts of pesticide exposure on fish conspecific interactions: A systematic review and meta-analysis

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Highlights

- Fish conspecific interactions are significantly reduced by pesticide exposure
- Fish courtship was the most vulnerable behaviour to pesticide exposure
- Triazole and organochlorine pesticides impact conspecific interactions most
- There is a taxonomic bias in the literature with most studies using zebrafish
- Future studies should consider a diverse range of species and pesticides

Abstract

The production of chemical pesticides poses a critical threat to aquatic ecosystems worldwide, with adverse effects evident even at sublethal concentrations. Historically, ecotoxicologists have ignored an organism's social context when investigating the effects of pesticide exposure and, instead, have tended to focus on individual-level impacts. Recently, however, there has been a growing interest in understanding the social impacts of pesticide exposure. Despite this shift, a holistic understanding of how pesticides impact conspecific interactions (i.e., social behaviour towards individuals of the same species) is lacking due to the multitude of behaviours, pesticides and species currently investigated. In this meta-analysis, we examine the effects of pesticide exposure on conspecific interactions in fish by using data collected from 37 studies on 31 pesticides and 11 species. Our results indicate that pesticide exposure generally reduces the expression of conspecific interactions, but it does not affect the variability of responses between individuals. Courtship behaviour was the most impaired, suggesting that pesticide exposure could weaken how matings are partitioned among individuals in a population. Triazoles and organochlorines were the most impactful pesticide classes for mean differences in behaviour, while triazoles and organophosphates had the greatest effects on response variability. These findings indicate that endocrine-disrupting pesticides can impact fish conspecific interactions, regardless of their chemical class. Unfortunately, there is a large taxonomic bias in the literature, with most studies using zebrafish as a model, which, in turn, provides scope for studies using a broader range of fish species. We found little statistical evidence of publication biases in our dataset and our results were validated by sensitivity analyses. Overall, our synthesis suggests that pesticides broadly reduce the expression of social behaviours, though effects vary across behaviours, pesticide types, and fish species.

Keywords:

behavior, sociality, shoaling, aggression, agrochemical, anthropogenic, pollution

Introduction

Chemical pollution caused by the continuous production and use of pesticides in agricultural systems is widely regarded as a leading threat to biodiversity (Tang et al., 2021). The increasing human reliance on pesticides has resulted in their detection in terrestrial and aquatic ecosystems globally (Bernhardt et al., 2017; Tang et al., 2021). Aquatic ecosystems are particularly vulnerable to pesticide contamination due to extreme sensitivity to anthropogenic stressors. Consequently, there has been increasing research effort to understand the impacts of pesticides on aquatic ecosystems (Islam et al., 2022; Morrison et al., 2024). To do so, ecotoxicologists routinely use fish species such as zebrafish (*Danio rerio*), Japanese medaka *(Oryzias latipes)*, and guppies *(Poecilia reticulata)*, as test subjects to assess the potential impacts of chemical exposure (Hong and Zha, 2019). In fact, fish are routinely used as models in ecotoxicology due to their importance in many aquatic ecosystems, in addition to their amenability to laboratory conditions (Choi et al., 2021).

While pesticide exposure at high concentrations can be lethal to aquatic organisms, at the concentrations currently detected in global surface waters, pesticides have been shown to have sublethal effects on behaviour and physiology (Morrison et al., 2024). A growing number of studies have specifically uncovered these sublethal impacts on fish behaviour (Morrison et al., 2024; Shuman-Goodier and Propper, 2016). Behaviour has garnered considerable interest as a potentially powerful biomarker in ecotoxicology and chemical risk assessments due to its critical link with an organism's physiological state (Bertram et al., 2024, 2022; Scott and Sloman, 2004; Wong and Candolin, 2015). Traditionally, ecotoxicologists have focused on the impacts of pesticides on individual behaviour by exposing, housing, or testing fish in isolation (Martin and McCallum, 2021; Michelangeli et al., 2022; Pyle and Ford, 2017). However, to fully understand the sublethal impacts of pesticide exposure at the population level and over evolutionary timescales, it is important to consider conspecific interactions—that is, behaviour between individuals of the same species (Bertram et al., 2022; Boughman et al., 2024; Hamilton et al., 2016; Köhler and Triebskorn, 2013; Michelangeli et al., 2022).

As a result, behavioural ecotoxicologists have recently shifted their focus on the impacts of pesticide exposure to fish conspecific interactions (Morrison et al., 2024). Such behaviours include aggression (Boscolo et al., 2018), collective movement (Shuman-Goodier and Propper, 2016), and courtship (Aulsebrook et al., 2020). However, examining pesticide exposure studies in isolation makes it difficult to capture the broader impacts of pesticides on conspecific interactions across various behaviours, pesticides, and species (Morrison et al., 2024). Furthermore, differences in study methodologies—including variations in behavioural assays used, dosages and durations of pesticide exposure, and the sex and source of fish—contribute to significant variation among studies, making generalised conclusions difficult (Morrison et al., 2024). Despite these challenges, no study has systematically evaluated the overall effects of pesticide exposure on fish-conspecific interactions or the extent to which methodological differences influence observed fish responses. These highlighted shortcomings have contributed to the growing demand for more evidence synthesis in behavioural ecotoxicology (Bertram et al., 2022).

Meta-analysis is the statistical aggregation of research results and is a powerful methodology to summarise evidence on a given topic (Gurevitch et al., 2018). Meta-analysis can, therefore, be used to effectively aggregate research results across different behaviours, pesticides and species. Meta-analysis can also be used to investigate how differences in study methodologies contributed to overall observed heterogeneity, capturing variation between studies that is not due to sampling error (Nakagawa et al., 2017). Whilst previous meta-analysis on the effects of pesticides on fish behaviour showed mean decreases in swim speed and activity (Shuman-Goodier and Propper, 2016), more recent meta-analyses of fish neuromuscular biomarkers have indicated that pesticide exposure can reduce the variability of physiological responses (Santana et al., 2022, 2021). This raises the question of whether pesticide exposure can reduce the variability of behaviours such as conspecific interactions. This consideration is especially relevant because phenotypic variation is fundamental to the process of natural selection. If pesticides affect this variation of behaviour, they could, in turn, have evolutionary consequences (Boughman et al., 2024).

Given the highlighted limitations in our understanding of the impacts of pesticides on fish behaviour due to significant methodological differences among studies, we conducted a phylogenetically controlled meta-analysis. This analysis synthesised the overall impacts of pesticide exposure on the mean and variability of conspecific interactions and examined whether methodological differences contribute to the observed responses. Our meta-analysis, preregistered at https://osf.io/hdjpq/ , aimed to address several predefined objectives. First, we investigated how pesticide exposure affects the mean and variability of fish-conspecific interactions across all studies, specifically whether there is an overall increase or decrease in these measures. Second, we examined the influence of behavioural characteristics, such as the type of behaviour measured and the assays used, on the mean and variability of conspecific interactions. Third, we explored how pesticide characteristics, including the specific pesticides, dosages, exposure durations, and solvents, impact these outcomes. Finally, we assessed how fish characteristics, such as species, source, and sex, influence the mean and variability of conspecific interactions.

Methodology

We preregistered the search strings, screening eligibility criteria and planned analyses prior to literature screening (see https://osf.io/hdjpq/). To be transparent on the completeness of reporting we provide a PRISMA-Eco Evo (O'Dea et al., 2021) checklist in Supplementary File 1. The PRISMA checklist was filled in by KM and reviewed by YY. All data, code, model outputs and additional information required to reproduce this study are provided at https://github.com/KyleMorrison99/fish_conspecific_behaviour_MA. We have also provided a detailed markdown file with all code required to reproduce the results https://kylemorrison99.github.io/fish_conspecific_behaviour_MA/. The reporting of the methodology followed MeRIT to improve author contributions' granularity and accountability (Nakagawa et al., 2023a). Additional details relevant to the methodology can be found in the Supplementary File 2.

Literature search strategy

To find relevant studies on the impacts of pesticide exposure on fish-conspecific interactions, we accessed Scopus, ISI Web of Science Core Collection, and PubMed on 01/03/2024. Additionally, we searched the grey literature using the Bielefeld Academic Search Engine (BASE) and ProQuest. All search strings are provided in full in Supplementary File 2. To augment the database search, KM conducted a backward/forward citation search on 6 relevant reviews already published on the topic (Bertram et al., 2022; Cally et al., 2019, 2019; Köhler and Triebskorn, 2013; Michelangeli et al., 2022; Saaristo et al., 2018; Shuman-Goodier and Propper, 2016; Söffker and Tyler, 2012). KM tested the sensitivity of the search against 10 benchmark papers identified independently of the search process using Google Scholar (Boscolo et al., 2018; Gusso et al., 2020; Hawkey et al., 2021; Jaensson et al., 2007; MacLaren, 2023; Saglio and Trijasse, 1998; Schmidel et al., 2014; Shenoy, 2012; Zaluski et al., 2022; Zhou et al., 2021).

Literature screening strategy

To screen for relevant literature, KM, supported by ML, MM, SO, RE, GM, JM, AB, and BW, developed a set of eligibility criteria (*Figure s1* for screening flowchart). The screening strategy followed a two-step approach: first, studies were screened based on abstract relevance, and second, by full-text relevance. To ensure thorough screening, all literature was reviewed in duplicate with each reviewer blind to the others decision (KM 80%, ML 13%, MM 13%, SO 13%, RE 13%, GM13%, JM 13%, AB 12%). Studies that were either author indicated "Yes" or "Maybe" at the abstract screening stage were included for full-text screening. For inclusion in the meta-analysis, both reviewers had to agree with a "Yes." Conflicts between reviewers at the full text screening stage were resolved through discussion, with a mediator (SN) present if required. All studies rejected at the full-text screening stage were provided with exclusion reasons *(Table s2)*. The literature screening was carried out using the screening software *Rayyan* (Ouzzani et al., 2016).

Data extraction

KM extracted data from all relevant studies, with 30% of the extracted data double-checked (10% each by YY, GM, and SN). For each study, we extracted a set of predefined variables following the preregistration (https://osf.io/hdjpq/). We have provided descriptions and full definitions of all variables in Supplementary File 2, Section *Data extraction variables*. In short, the extracted variables included behavioural characteristics—such as the behaviours measured (aggression, courtship, social attraction, and collective movement) and the behavioural assays used (zone, count, entries); pesticide characteristics—including the pesticide used for exposure, its dosage, duration, and the solvent employed; and species characteristics—such as the species exposed, their sex, and the source of the fish. All statistical variables needed to calculate effect size estimates were extracted from text and tables when available. Otherwise, we extracted data from figures using R packages S*hiny Digitise* and *Meta Digitise* (Pick et al., 2019). When raw data or individual points from figures were provided, we calculated means, errors, and sample sizes from the raw data. We imputed standard deviations of effect size estimates when it was missing by using the mean-variance relationship identified (Figure s14) (Lajeunesse, 2016). To enrich the insights provided during the data extraction we incorporated a systematic evidence map approach to visualise study characteristics (Yang et al., 2023).

Effect size calculations

We estimated the impacts of pesticides on both the magnitude and variability of conspecific interactions. To measure magnitude and variability we used the response ratio (RR) and the variation ratio (VR), respectively. To approximate normality, both effect size estimates were logarithmically transformed. We defined the two effect size estimates along with their sampling variances as follows:

Response ratio (see Lajeunesse, 2015)

$$lnRR = ln\left(\frac{\bar{x}_{treatment}}{\bar{x}_{control}}\right) + \frac{1}{2}\left(\frac{s^2{}_{treatment}}{n_{treatment}\ \bar{x}^2{}_{treatment}} - \frac{s^2{}_{control}}{n_{control}\ \bar{x}^2{}_{control}}\right), (1)$$

$$s^2{}_{lnRR} = \frac{s^2{}_{control}}{n_{control}\ \bar{x}^2{}_{control}} + \frac{s^4{}_{control}}{2n^2{}_{control}\ \bar{x}^4{}_{control}} + \frac{s^2{}_{treatment}}{n_{treatment}\ \bar{x}^2{}_{treatment}} + \frac{s^4{}_{treatment}}{2n^2{}_{treatment}\ \bar{x}^4{}_{treatment}}, (2)$$

Variation ratio (see Senior et al., 2020)

$$lnVR = ln\left(\frac{s_{treatment}}{s_{control}}\right) + \frac{1}{2}\left(\frac{1}{n_{\mathrm{control}} - 1} + \frac{1}{n_{\mathrm{treatment}} - 1}\right), (3)$$

$$s^2_{\mathrm{lnVR}} = \frac{1}{2}\left(\frac{n_{\mathrm{control}}}{(n_{\mathrm{control}} - 1)^2} + \frac{n_{\mathrm{treatment}}}{(n_{\mathrm{treatment}} - 1)^2}\right), (4)$$

Where $\bar{x}_{treatment}$ and $\bar{x}_{control}$ are the (sample) mean of conspecific interaction for the treatment and control, respectively; $s_{control}$ and $s_{treatment}$. are the (sample) standard deviations (SDs), $n_{control}$ and $n_{treatment}$ are the corresponding sample sizes.

Statistical modelling summary

All statistical modelling was conducted by KM (checked by SN and YY). To analyse the effect size estimates, we used multi-level meta-analysis models with a sampling variance-covariance matrix (Nakagawa et al., 2023c). The t-distribution was used to compute the test statistics and confidence intervals for the fixed effects, and the restricted maximum likelihood (REML) was used as the model estimator. The constructed models accounted for four types of statistical dependency: 1) the dependency of multiple effect sizes per study, pesticide and species, 2) different levels of phylogenetic relatedness between species, 3) the correlation of errors due to repeated behavioural measurements from the same set of individuals and, 4) multiple treatment groups being compared to a single control group (i.e., shared control between treatments). To quantify heterogeneity (i.e., variance not due to sampling error) we calculated the total heterogeneity ${I^2}_{total}$, which indicates the total variance excluding sampling variance. Then, we decomposed the ${I^2}_{total}$ into the different random effects including between study, between observation, between pesticide and between species (i.e., ${I^2}_{study}$, ${I^2}_{observation}$, ${I^2}_{pesticide}$ and ${I^2}_{species}$). Robust-variance estimation was not used because pesticides and species are crossed random effects not nested random effects (Y. Yang et al., 2023). To assess whether effect size estimates were influenced by predefined predictor variables we constructed a series of meta-regression models. The marginal R^2 was used to quantify the proportion of heterogeneity explained by each moderator (Nakagawa and Schielzeth, 2013). We have provided the model parameters for both intercept-only and predictor models in Supplementary File 2.

Model selection and multi-modal inference

To test the robustness of the results obtained from the predictor models we conducted model selection and multi-model inference (Cinar et al., 2021). This was completed by, fitting 64 models with all possible combinations of predictor variables. We then assessed their AICc values to select the best models whose AICc were <2 units larger than the lowest AICc (Grueber et al., 2011). We then evaluated the importance of the predictor variables by considering all 64 models' Akaike weights. Each of the 64 models had the same random effects structure as the predictor models but were fitted using maximum likelihood rather than REML to allow model comparison (Cinar et al., 2021).

Publication bias, time lag bias and sensitivity analysis

Publication bias refers to the unequal likelihood of significant findings being published when compared to nonsignificant results, thus creating a bottleneck of underrepresented study findings which, in turn, may potentially lead to unfounded conclusions. We visually inspected the relationship between model residuals and the standard error using funnel plots. This methodology assumes no heterogeneity and, thus, should not be used in isolation. We then performed a multilevel Egger's regression to test the symmetry of the funnel plot using sampling variance as a moderator. Time-lag bias refers to the cases when earlier published studies tend to show larger effect size estimates with smaller sample sizes. To assess the potential time-lag bias, we implemented a multi-level meta-regression with publication year as a moderator. Publication bias is likely only an issue for mean differences because studies did not explicitly test for differences in variability (Yang et al., 2022). Therefore, all publication bias assessments were only conducted for *lnRR*. To further assess the robustness of results, we conducted four sensitivity analyses. We first conducted a leave-one-out cross-validation, where one study, pesticide or species was excluded from the dataset, and the intercept-only model was rerun (see Supplementary File 2 for formulas). Second, we reanalysed the intercept-only model using an alternative variance-covariance matrix under different assumptions about non-independence. Specifically, when it was unclear, we considered two scenarios: assuming that the exposure group comprised the same individuals across different behaviours (resulting in dependent estimates), or assuming they were different individuals (resulting in independent estimates) (Noble et al., 2017). Third, we reanalysed the intercept-only model without the imputed error estimates. Fourth, we conducted an alternative intercept-only analysis using lnCVR instead of lnVR to re-estimate response variability.

Statistical analysis software

All data analysis was conducted on the R Statistical Environment version 4.2.1 (R Core Team, 2022) using RStudio build 576 (RStudio Team, 2022). The phylogenetically controlled multi-level meta-analysis and meta-regression models were implemented using the *rma.mv* function in the *metafor* package (Viechtbauer, 2010). To infer the phylogenetic relatedness, we constructed a phylogenetic tree using the *Open Tree of Life* implemented using the *rotl* package (Michonneau et al., 2016). The branch length was calculated using the Grafen's method and we implemented using the *ape* package (Paradis et al., 2024). To construct the variance- covariance sampling matrix we use the *vcalc* function in *metafor* assuming a constant variance of $\rho = 0.5$. All visualisations of the models were constructed using *ggplot2* (Wickham, 2016) and the *orchaRd* 2.0 package (Nakagawa et al., 2023b).

Deviations from preregistration

While we closely followed our preregistration (see https://osf.io/hdjpq/), we made several minor adjustments and improvements. First, to examine differences in variability between control and treatment groups, we chose *lnVR* as the effect size measure instead of the originally proposed *lnCVR*. We made this selection because the dimensions of the measurements and the true mean-variance relationship are unknown, and *lnVR* clearly demonstrates variation differences irrespective of the mean (Pélabon et al., 2020). Second, although we initially planned to include phylogeny in all models, we ultimately limited its inclusion to intercept-only models based on our findings. Third, to improve our analysis, we introduced additional variables during data extraction and analysis. Specifically, we included two extra columns to indicate whether studies used a control solvent and whether they employed a zone-based or count-based assay. In addition, we added an alternative variance-covariance matrix because cohort identification was often unclear across studies.

Results

Summary of literature

We collected 449 effect sizes from 37 experimental studies involving 31 pesticides and 11 species (Figure 1a). The behaviours measured in response to pesticide exposure were social attraction (24.6%, 110 effect size estimates), collective movement (21.2%, 95 effect size estimates), courtship (20.1%, 90 effect size estimates) and aggression (34.1%, 153 effect size estimates) (Figure 1b). For species characteristics, we found that the most widely studied (73%, 327 effect size estimates) model species was zebrafish (Figure 1c). In addition, many studies (51%, 232 effect size estimates) used fish of both sexes without distinguishing between them (Figure s3). The fish were most often obtained directly from commercial suppliers (44%, 197 effect size estimates, Figure s4). For pesticide exposure characteristics, we found the most common pesticides investigated were deltamethrin (15.4%, 69 effect size estimates) and atrazine (12.3%, 55 effect size estimates) (Figure s5). We found a range of dosages (median = 12 ug/L, 1st quartile = 1 ug/L, 3rd quartile = 500 ug/L; Figure s6) and durations (median = 336 hours, 1st quartile = 96 hours, 3rd quartile = 960 hours; Figure s7) were used in the pesticide exposure. Furthermore, many studies did not use a chemical solvent (29.5%, 132 effect size estimates) or, did not report whether a chemical solvent was used (28.1%, 125 effect size estimates). However, when a solvent was reported, the most widely used was Dimethyl sulfoxide (DMSO)(31.5%, 141 effect size estimates) (Figure s8).

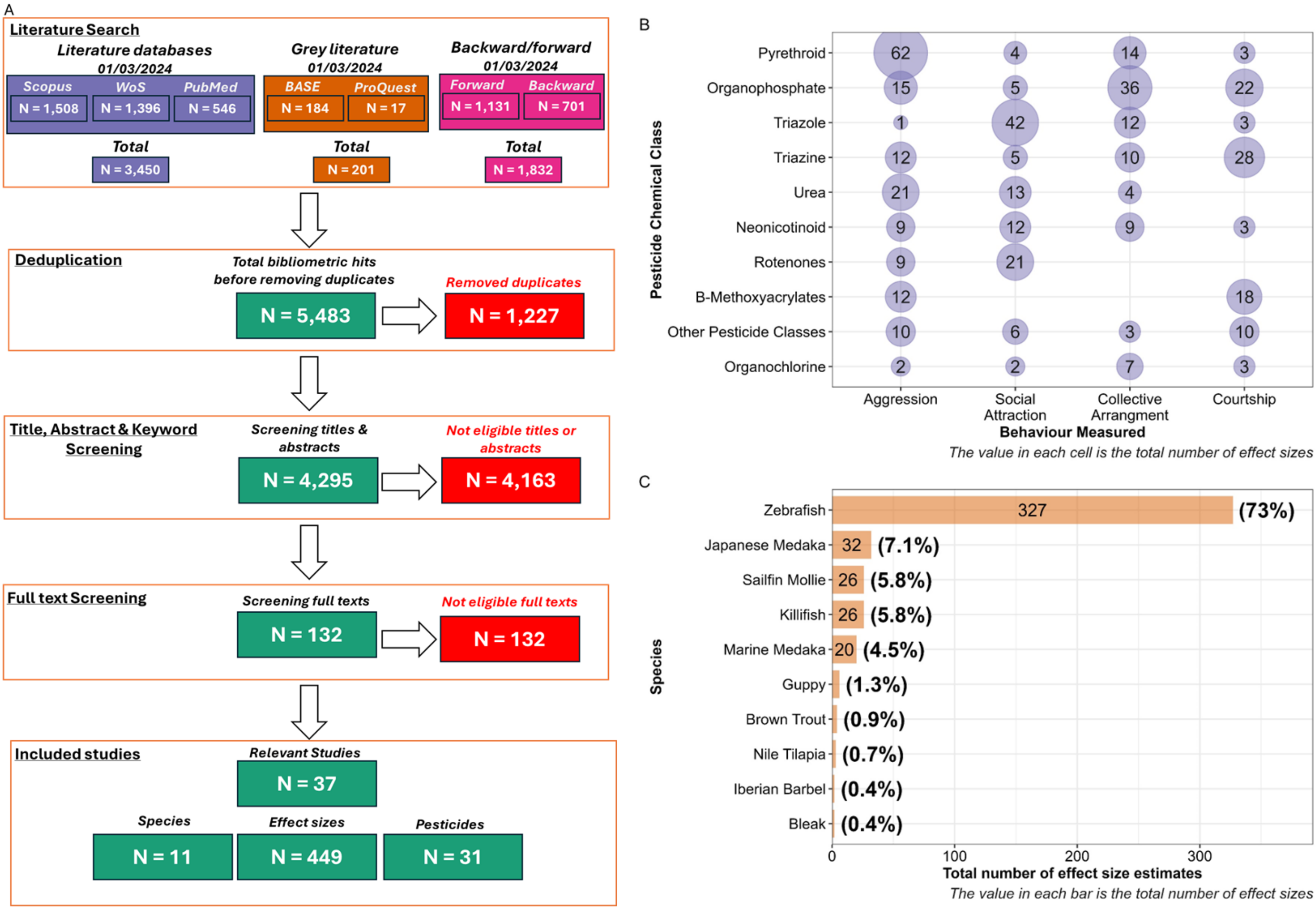


Figure 1. (A) PRISMA flowchart summarizing the search methods used and the number of studies excluded at each step. (B) a circle plot showing the total number of effect sizes for each pesticide chemical class per behaviour measured. (C) a bar plot showing the total number of effect sizes for each species.

Overall effect on mean and variability

Pesticide exposure significantly decreased conspecific interactions by 23.4% on average ($\beta_{\ln RR}$ = -0.2669, 95% confidence interval (CI) = [-0.4868, -0.0471], t_{447} = -2.3862, p = 0.0174; Figure 2A). In contrast, we found that pesticide exposure tended to not impact the variability of conspecific interactions with a decrease on average of 7.02% ($\beta_{\ln VR}$ = -0.0728, CI = [-0.1549, -0.0094], t_{447} = - 1.7409, p = 0.0824; Figure 2B). The relative data heterogeneity was high for *lnRR* effect size estimates (I^2_{total} = 97.42%) and moderate for lnVR (I^2_{total} = 70.58%). We explored the contribution of all the included random effects for both *lnRR* and *lnVR*. We found that $I^2{}_{study}$ = 28.69%, $I^2{}_{observation}$ = 31.34%, $I^2{}_{pesticide}$= 4.34% and $I^2{}_{species}$ = 33.05% for lnRR; whilst $I^2{}_{study}$ = 3.24%, $I^2{}_{observation}$ = 67.34%, $I^2{}_{pesticide}$= <0.001% and $I^2{}_{species}$ = <0.001% for *lnVR*.

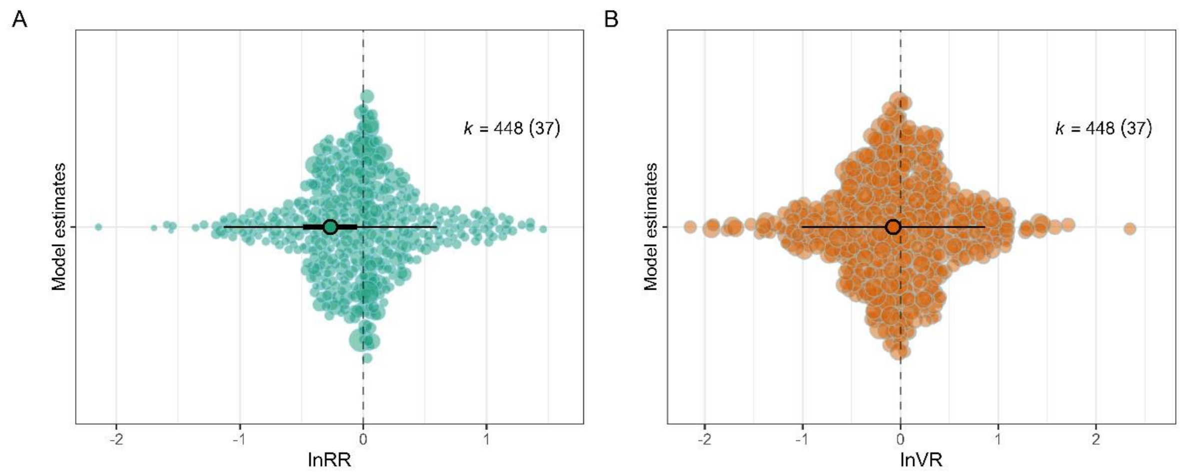


Figure 2. Impacts of pesticide exposure on fish conspecific interactions. The model estimates the average effects of pesticide exposure on conspecific interactions in fish. (A) shows the mean difference between control and treatment groups on a logarithmic

scale (lnRR), where negative values indicate a reduction in conspecific behavioural activity. (B) shows the difference in variances between control and treatment groups, also on a logarithmic scale, where negative values suggest a reduction in the inter-individual variability of conspecific behavioural activity (lnVR). Shorter-thicker whiskers represent 95% confidence intervals, while longer-thinner whiskers indicate 95% prediction intervals. 'k' represents the number of effect sizes, and the number of studies is in brackets. Each circle corresponds to an effect size, with its size scaled according to precision (inverse sampling error variance).

Impacts on conspecific behaviour characteristics

The conspecific interaction measured in response to pesticide exposure played a significant role in moderating the mean and an insignificant role in moderating variability changes (*lnRR*: $F_{4,444}$ = 7.1848, p <0.0001, R^2_{marginal} = 0.07; *lnVR*: $F_{4,444}$ = 1.3083, p = 0.266, R^2_{marginal} = 0.02). For mean differences, we found that courtship significantly decreased in response to pesticide exposure on average by 34.82% ($\beta_{\text{lnRR\_courtship}}$ = -0.4280, CI = [-0.6585, -0.1976], t_{444} = - 3.6501, p = 0.003; Figure 3A). On the other hand, aggression, sociality and collective movement was not significantly impacted by pesticide exposure ($\beta_{\text{lnRR\_aggression}}$ = -0.1251, CI = [-0.3531, -0.1030], t_{444} = - 1.0779, p = 0.2817; $\beta_{\text{lnRR\_collective\_behaviour}}$ = -0.1788, CI = [-0.4183, 0.0607], t_{444} = - 1.4675, p = 0.1430; ($\beta_{\text{lnRR\_sociality}}$ = -0.1530, CI = [-0.3886, 0.0825], t_{444} = - 1.2768, p = 0.2023; Figure 3A). For variational differences, we found that none of the behaviours had a significant difference between control and treatment groups ($\beta_{\text{lnVR\_courtship}}$ = -0.1674, CI = [-0.3718, 0.0370], t_{444} = -1.6092, p = 0.1083; $\beta_{\text{lnVR\_aggression}}$ = -0.0470, CI = [-0.2554, -

0.1613], t_{444} = - 0.4436, p = 0.6476; $\beta_{\mathrm{lnVR\_collective\_behaviour}}$ = -0.0481, CI = [-0.2554, 0.1613], t_{444} = - 0.4135, p = 0.6794; $\beta_{\mathrm{lnVR\_sociality}}$ = 0.0743, CI = [-0.1505, 0.2990], t_{444} = - 0.6493, p = 0.5165; Figure 3B). There was no significant difference in magnitude or variability between zone-based assays and count-based assays ($\beta_{\mathrm{lnRR\_assay\_contrast}}$ = - 0.0235, CI = [-0.1430, 0.959], t_{278} = - 0.3879, p = 0.6984, Figure 3C; $\beta_{\mathrm{lnVR\_assay\_contrast}}$ = 0.0523, CI = [-0.1104, 0.2149], t_{278} = - 0.6328, p = 0.5274, Figure 3D).

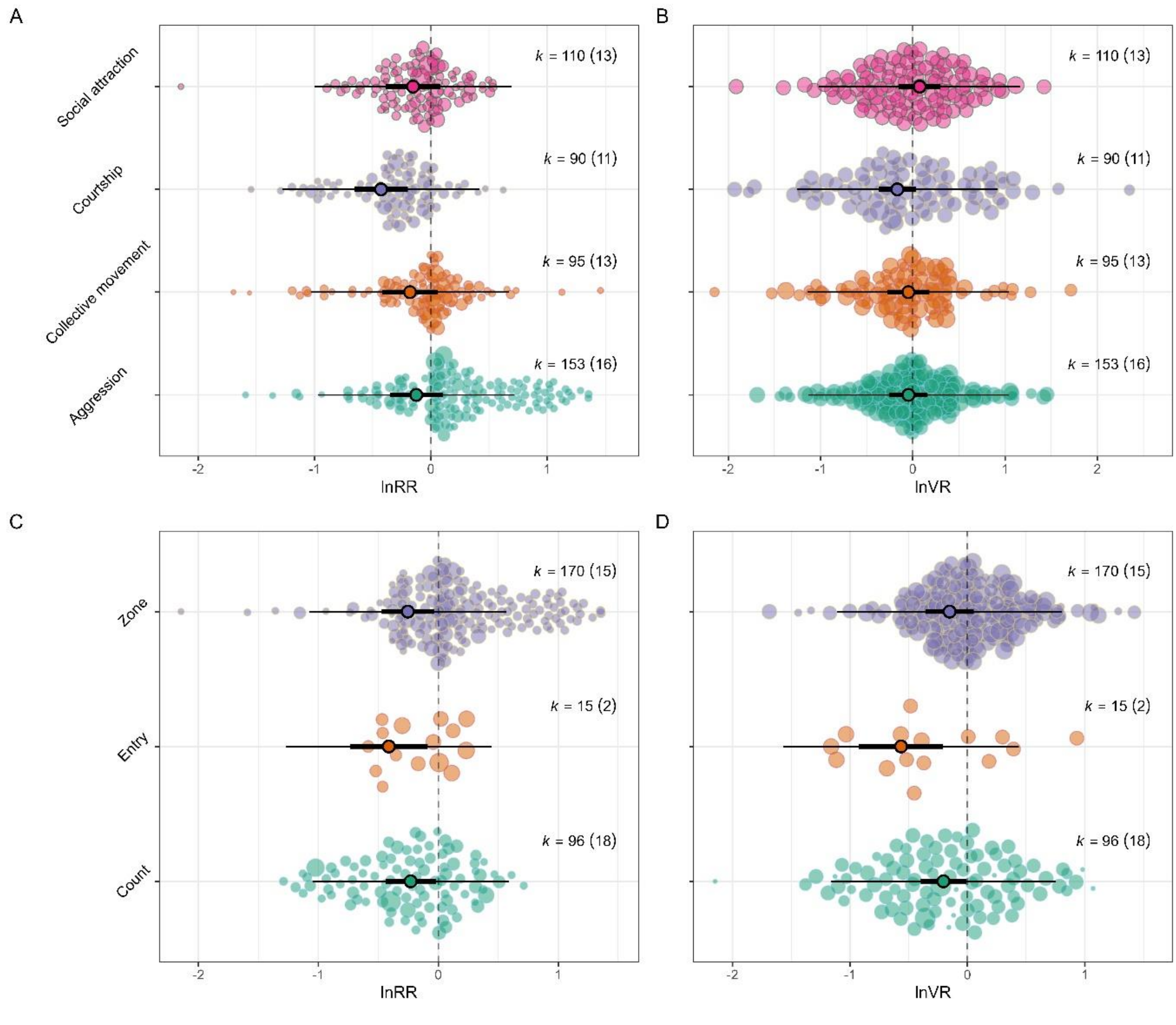


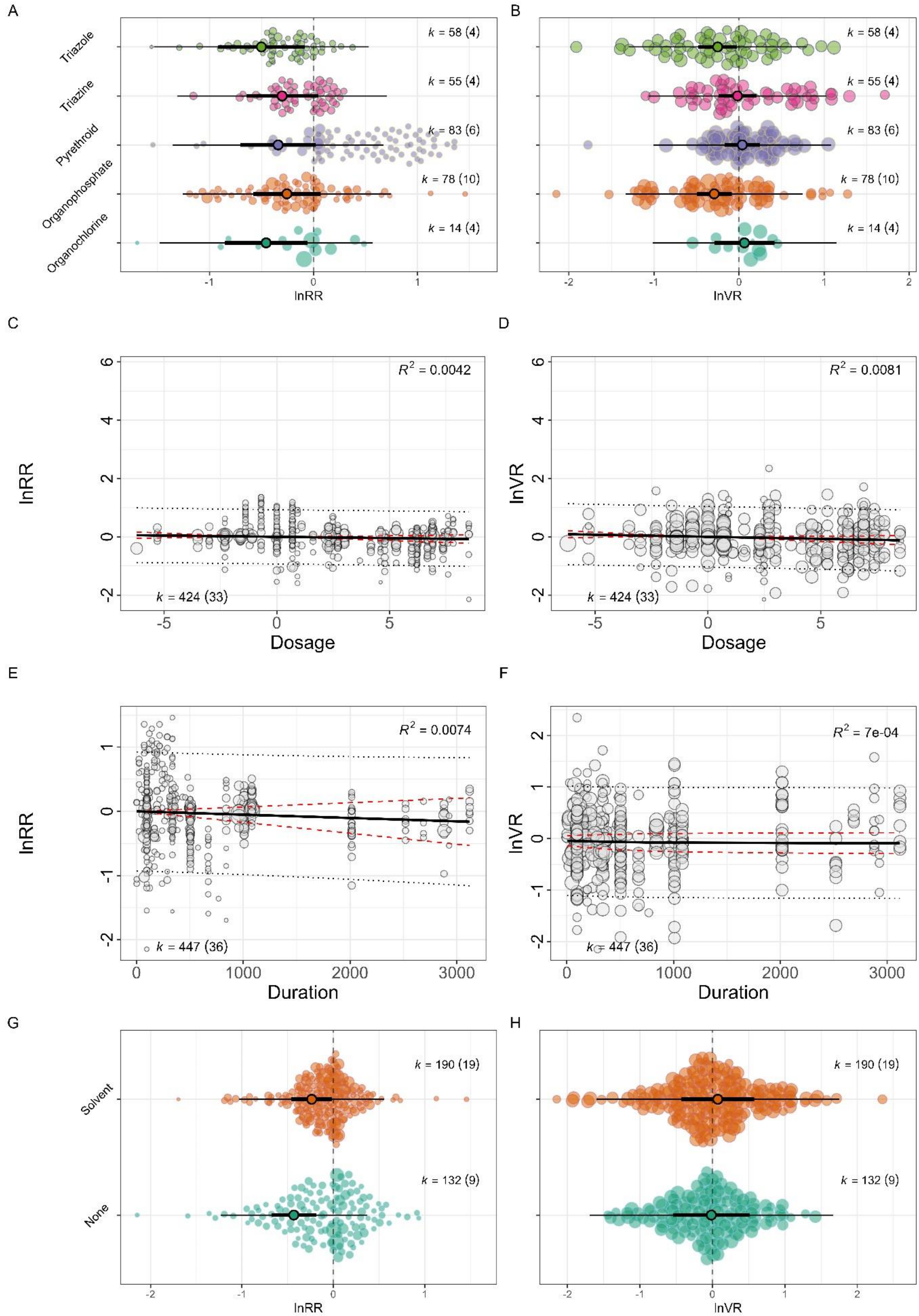
Figure 3. The moderating effects of behaviour measured: Social Attraction, Courtship, Collective Movement and Aggression on (A) response magnitude of conspecific interaction, and (B) response variability of conspecific interactions, followed by the

moderating effects of assay type used: Zone, Entry and Count on (C) response magnitude of conspecific interaction, and (D) response variability of conspecific interactions. The model estimates were obtained using a uni-moderator meta-regression. Refer to Supplementary File 2 for full definitions of all extracted variables. The remaining details are the same as in Figure 2.

Impacts of pesticide characteristics

We found no significant differences in mean responses across pesticide classes, but there was a significant difference in response variability (*lnRR*: $F_{14,434}$ = 1.3108, p = 0.1969, R^2 = 0.1103; *lnVR*: $F_{14,434}$ = 2.0818, p = 0.0119, R^2 = 0.10). For mean differences, we found that organochlorines and triazoles significantly decreased interactions with conspecifics ($\beta_{\mathrm{lnRR\_organochlorine}}$ = -0.1674, CI = [-0.3718, 0.0370], t_{435} = -2.2570, p = 0.0245; $\beta_{\mathrm{lnRR\_triazole}}$ = -0.5014, CI = [-0.9188, -0.0841], t_{435} = - 2.3614, p = 0.0186, Figure 4A). For variability differences, we found that organophosphates and organochlorines led to a significant decrease in variability ($\beta_{\mathrm{lnVR\_organophosphate}}$ = -0.2923, CI = [-0., 0.4992, -0.0855], t_{435} = -2.2778, p = 0.0057; $\beta_{\mathrm{lnRR\_triazole}}$ = -0.2512, CI = [-0.4785, -0.0238], t_{435} = - 2.1709, p = 0.0305, Figure 4B). For moderating effects of dosage, we found no significant relationship between the dosage of pesticide exposure and the effect on mean or the variability of conspecific interactions ($\beta_{\mathrm{lnRR\_dosage}}$ = -0.0090, CI = [-0.0254, 0.0074], t_{423} = -1.0780, p = 0.2817, R^2_{marginal} = 0.0042, Figure 4C; $\beta_{\mathrm{lnVR\_dosage}}$ = -0.0140, CI = [-0.0323, -0.0042], t_{423} = - 1.5142, p = 0.1307, R^2_{marginal} = 0.0081; Figure 4D). Likewise, for moderating effects of duration we found no significant relationship between duration of pesticide exposure and the mean or the variability of behaviours measured ($\beta_{\mathrm{lnRR\_duration}}$ = -0.0001, CI = [-0.0002, 0.0001], t_{446} = -0.8544, p = 0.3933,

$R^2_{marginal}$ = 0.0074, Figure 4E; $\beta_{lnVR\_duration}$ = -0.0112, CI = [-0.0366, -0.0143], t_{423} = -0.8625, p = 0.3889, $R^2_{marginal}$ = 0.0007; Figure 4F). We found a weak yet significant difference in mean estimates between studies that used a control solvent and those that did not, but no significant difference in the variability estimates ($\beta_{lnRR\_solvent\_contrast}$ = 0.1958, CI = [-0.0670, 0.3247], t_{320} = 2.9908, p = 0.0006, Figure 4G; $\beta_{lnVR\_solvent\_contrast}$ = 0.0893, CI = [-0.1013, 0.2798], t_{320} = 0.9218, p = 0.3573, Figure 4H).



A
B
Triazole
Triazine
Pyrethroid
Organophosphate
Organochlorine
k = 58 (4)
k = 55 (4)
k = 83 (6)
k = 78 (10)
k = 14 (4)
lnRR
lnVR
C
D
R^2 = 0.0042
R^2 = 0.0081
k = 424 (33)
Dosage
E
F
R^2 = 0.0074
R^2 = 7e-04
k = 447 (36)
Duration
G
H
Solvent
None
k = 190 (19)
k = 132 (9)

Figure 4. The moderating effects of pesticide chemical class on (A) response magnitude and (B) response variability in conspecific interactions. Only chemical classes with more than three studies are included here; the complete plot with all chemical classes is available in the Supplementary File 2 (Figure s18). Following this, we show the (C & D) moderating effects of dosage (ug/L, axis presented on the logarithmic scale) and (E & F) duration (hours) on both the mean and variability of the response. Minimal variance explained by dosage and duration is indicated by the R^2 values. Finally, the moderating effects of solvent use on response magnitude (G) and variability (H) are presented, with separate comparisons for conditions with and without solvents. Model estimates were obtained using univariate moderator meta-regressions. Further details are consistent with those provided in Figure 2.

Species sensitivities

Overall, we found that the species of fish did not play a significant role in moderating the impacts on the mean or the variability of response (*lnRR*: $F_{10,438}$ = 0.9211, p = 0.0723, R^2 = 0.11; *lnVR*; $F_{10,438}$ = 0.9211, p = 0.5134, R^2 = 0.07). However, it is important to note, many species are understudied with limited effect size estimates (Figure 1B). Therefore, confidence intervals are large, and precision is low for most species (Figure 1C, Figure 5). In terms of sex of fish, we found no significant difference between female and male fish for both effects on mean and variability ($\beta_{\mathrm{lnRR\_sex\_contrast}}$ = -0.0809, CI = [-0.2429, 0.0811], t_{70} = -0.9960, p = 0.3227; $\beta_{\mathrm{lnVR\_sex\_contrast}}$ = 0.1204, CI = [-0.217 4, 0.4582], t_{70} = 0.7107, p = 0.4797 Likewise, we did not find a significant difference in the mean or the variance between wild collected fish and laboratory bred/commercially purchased fish

($\beta_{lnRR\_source\_contrast}$ = -0.4703, CI = [-1.5404, 05998], t_{396} = -0.8663, p = 0.3873; $\beta_{lnVR\_source\_contrast}$ = -0.0490, CI = [-0.4265, 0.3285], t_{396} = -0.2560, p = 0.7982).

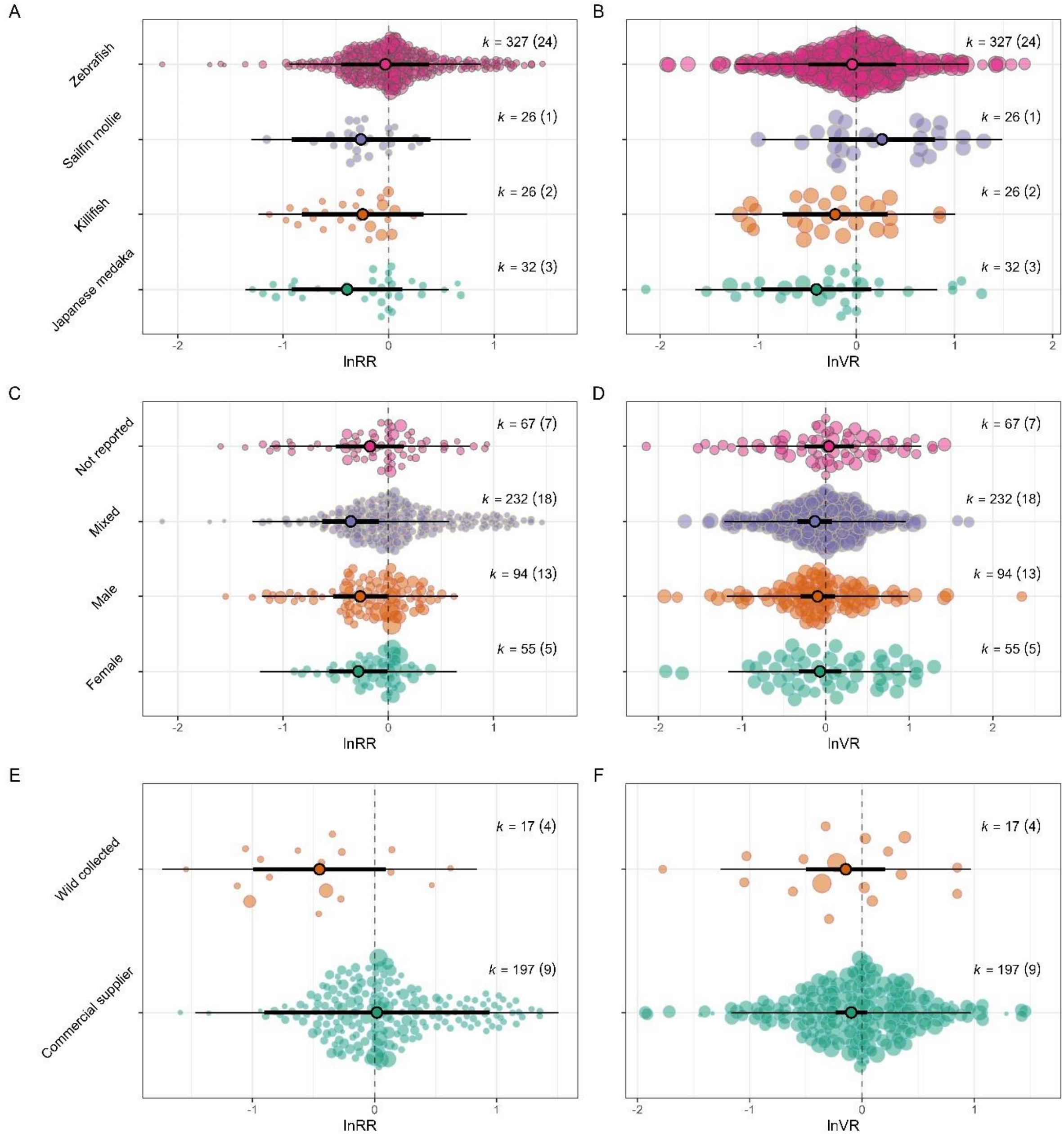


Figure 5. The moderating effects of species on conspecific interactions, showing (A) response magnitude and (B) response variability. The figure is filtered to include only species with more than 15 effect size estimates; the complete plot, including all species, is provided in the Supplementary File 2 (Figure s19). Following this, we show

the moderating effects of sex on (C) response magnitude and (D) response variability. Finally, we present the moderating effects of fish source type on (E) response magnitude and (F) response variability. Model estimates were obtained using univariate moderator meta-regressions. The remaining details are the same as those in Figure 2.

Model selection and multimodal inference

The model, including all moderators, explained 71.4% of variation in mean differences and 30.5% of variation in variability differences. Model selection revealed that the type of behavioural assay was an important moderator for both mean and variability estimates (Figure 6). The model with the lowest AICc (225.3307) for estimating mean differences included behavioural assay, pesticide chemical class, species and sex of fish and had an 18.7% probability of being the best model. Whilst the model with the lowest AICc (425.4661) for estimating variability differences included behaviour assay, pesticide chemical class and species, and had a 21.5% probability of being the best model.

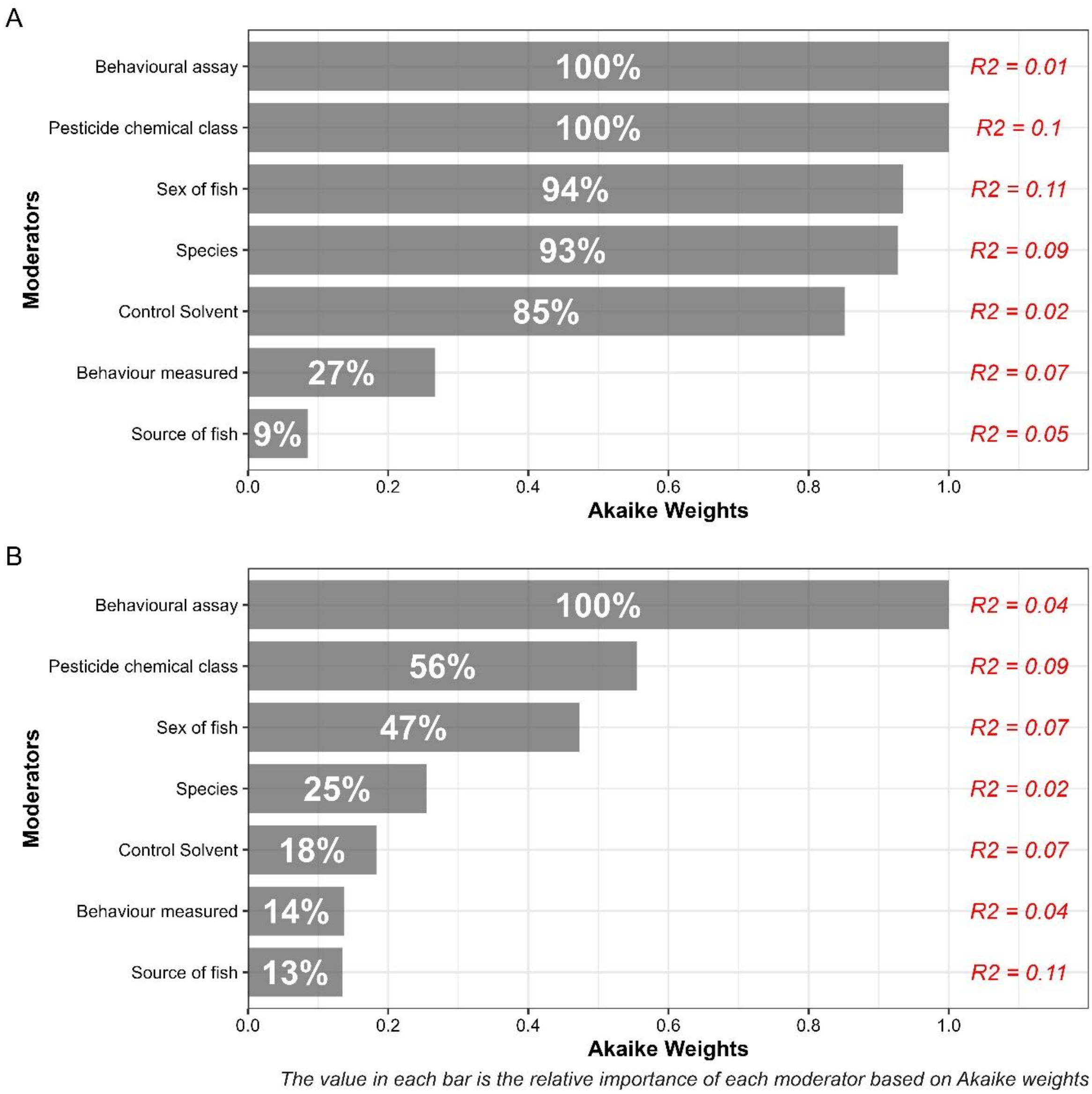


Figure 6. The relative importance of tested moderator variables based on Akaike weights calculated from the Akaike Information Criterion (AIC) for (A) lnRR and (B) lnVR. The importance of each moderator variable was assessed across 64 candidate models by summing the Akaike weights of all models in which the variable appeared. These Akaike weights approximate the probability that a given model is the best among the candidate set, assuming equal prior probabilities for all models. Additionally, the marginal R^2

which indicates the proportion of variance explained, was estimated using the uni-moderator model with the corresponding moderator variable as the fixed effect.

Publication bias, time-lag bias and sensitivity analysis

We found minimal evidence of publication bias (i.e., no bias towards the publication of significant results) detected by visual inspection of the funnel plot (Figure s20) and Egger's regression analysis ($\beta_{\mathrm{lnRR\_sampling\_error}}$ = -0.0809, CI = [-4642, 0.3025], t_{446} = -0.4145, p = 0.6787; Figure s21) and we found no time-lag bias in effect sizes over time ($\beta_{\mathrm{lnRR\_publication\_year}}$ = -0.0582, CI = [-0.0303, 0.1467], t_{446} = 1.2930, p = 0.1967; figure s22). We further investigated the robustness of our results through four sensitivity analyses. Excluding individual studies, species, or pesticides from the models had little influence on the magnitude of results. However, we found that excluding some species or pesticides changed the significance of results (Figure s23-28). Furthermore, using an alternative variance-covariance structure with a different assumption of non-independence had little impact on the outcomes ($\beta_{\mathrm{lnRR\_alternative\_vcv}}$ = -0.2655, 95%, CI = [-0.4839, -0.0470], t_{447} = - 2.3886, p = 0.0173, Figure s29A; $\beta_{\mathrm{lnVR\_alternative\_vcv}}$ = -0.0799, 95%, CI = [-0.2557, -0.00959], t_{447} = - 0.8933, p = 0.3722; Figure s29B). Last, we found that excluding the imputed error estimates had little influence on the analysis conclusion ($\beta_{\mathrm{lnRR\_no\_imputed}}$ = -0.2460, 95%, CI = [-0.4719, -0.0201], t_{443} = - 2.1402, p = 0.0329. Figure s30A; $\beta_{\mathrm{lnVR\_no\_imputed}}$ = -0.0239, 95%, CI = [-0.2986, -0.2509], t_{443} = -0.1706, p = 0.8646; Figure s30B).

Discussion

In response to growing evidence that pesticide exposure affects fish behaviour, this study aimed to quantify its overall impact on conspecific interactions and identify how these effects vary between different behaviours, pesticides, and species studied. Here, we conducted a meta-analysis, synthesising evidence from 37 studies involving 31 pesticides and 11 species, offering the first cross-chemical and cross-species quantification of the impacts of pesticide exposure on fish-conspecific interactions. Overall, we found that pesticide exposure significantly reduced fish conspecific interactions by an average of 23.4%, while the variability of responses was not significantly affected (a 7% change on average). The overall heterogeneity for both the mean and the variability of response was large, and both within-study differences and the study species contributed greatly to this heterogeneity. The sensitivity analysis revealed that the results of the meta-analysis were robust, and little statistical evidence for publication bias in our dataset. This overall decrease in conspecific interactions aligns with other syntheses that have quantified significant declines in fish activity (Shuman-Goodier and Propper, 2016) and neuromuscular function (Santana et al., 2021) due to pesticide exposure. This suggests that impairing behaviour at the muscular control level can reduce fish's ability to perform behaviours, with likely knock-on consequences for their social competence and, hence fitness (Taborsky and Oliveira, 2012).

Impacts on conspecific behaviour characteristics

We found that pesticide exposure significantly and consistently decreased courtship behaviours in fish. In contrast, aggression, sociality, and collective behaviours exhibited inconsistent changes across studies; some reported increases while others observed decreases, reflecting high heterogeneity in the data. This variability may stem from methodological differences between and within studies, as shown by the moderate heterogeneity in the intercept-only model (see Figure 1). The observed reduction in courtship behaviours may result from several mechanisms. First, endocrine-disrupting pesticides, such as organochlorines and organophosphates, can interfere with hormonal functions, decreasing the motivation of fish to engage in courtship (Sárria et al., 2011). Second, pesticide exposure can impair sensory perception and cognitive abilities, making it more difficult for fish to locate or identify potential mates (Bridi et al., 2017). Third, pesticides can reduce neuromuscular function and deplete energy reserves, leading fish to allocate less energy to courtship activities (Santana et al., 2021; Shuman-Goodier and Propper, 2016).

The complex impacts of pesticide exposure on fish social attraction and collective movement may be due to reduced activity in response to pesticides. Subsequently, this may decrease social responses in some contexts (Shuman-Goodier and Propper, 2016), while heightened anxiety may enhance social attraction and collective movement in others (Faria et al., 2021). Similarly, we found that aggression can both increase and decrease under different pesticide exposures. This may be due to pesticides acting antagonistically with androgens or synergistically with oestrogens, which, in turn, may decrease aggression, whereas androgen-synergistic pesticides may increase aggression (Tomkins et al., 2017). That said, it is also possible that these differences could be due to alternative non-endocrine pathways, such as damage caused to tissues or receptors or reducing energy levels (Rohani, 2023). The observed decrease in courtship behaviour suggests that even sublethal concentrations of pesticides can affect the likelihood of exposed individuals successfully attracting mates (Boughman et al., 2024) and may alter how matings are partitioned among individuals in a population (Saaristo et al., 2018; Wong and Candolin, 2015). The multi-directional effects on sociality, collective behaviour, and aggression underscore the complexity of pesticide impacts on fish conspecific interactions.

Impacts of pesticide characteristics

Our analysis reveals that organochlorine and triazole pesticides exert the most significant detrimental effects on conspecific interactions. Both pesticide classes possess endocrine-disrupting properties and are known to affect the hypothalamic-pituitary-gonadal axis (Martyniuk et al., 2020; Taxvig et al., 2008). This suggests that endocrine-disrupting chemicals can influence fish-conspecific interactions (Söffker and Tyler, 2012). However, more research is required on a diverse range of chemicals other than pesticides to further investigate the impacts and mechanisms of endocrine-disrupting chemicals on fish-conspecific interactions (Husak et al., 2009).

We also discovered that organophosphates and triazoles can reduce behavioural variability among individual fish, indicating that some pesticides may make fish behaviours more predictable. This finding aligns with previous meta-analyses that reported a reduction in variability of fish physiological biomarkers following pesticide exposure (Santana et al., 2022, 2021). We found that there was high variation in the impacts between different chemical class, emphasising the need for a broad range of pesticides to be studied. Concerningly, we found that some of the most disruptive pesticides, such as the carbamates, remain understudied and, thus, under-represented in this evidence base (see Figure s18).

In terms of control solvents, we surprisingly found that there was a significant difference between studies using a control solvent and those that did not. This indicates that the solvents being used may have an influence on the conspecific interactions being measured and, thus, could mask the impacts of the pesticide exposure. Therefore, control solvents, as well as the concentrations of solvents used, must be carefully selected to ensure they do not influence the outcomes being measured (Bertram et al., 2024). Consequently, we suggest solvents should not be used unless necessary and, in cases where their use is required, it would be worthwhile to include control groups, both with and without the solvent, to test for the potential impact of the solvent itself (Green and Wheeler, 2013). Hence, it is important for future studies to consider a broad range of pesticides, as well as study characteristics, such as the solvents used (Bertram et al., 2024)

Species sensitivities and characteristics

We found that there were no significant differences between species. However, species differences did account moderately for the heterogeneity for both mean and variational differences. Differences in species sensitivity can arise for multiple reasons. First, species vary in their overall frequency or reliance on social behaviours. Species that are more social are therefore expected to be more likely to experience pesticide-induced alterations to their conspecific interactions. Second, species-specific responses to pesticides may be caused by differences in their general sensitivity to environmental change, where some species may be more robust and better physiologically equipped to handle contaminants than others (Nickisch Born Gericke et al., 2022). However, as mentioned previously, the current evidence is based on only a small handful of study species, with most research having been conducted on zebrafish. Therefore, the inability to detect species differences may simply be due to the scant research that has been done on species with a broader range of social structures.

We did not find differences between males and females in their responses to pesticide exposure. This is despite evidence that the impacts of chemical exposure can be sex-specific in the case of other toxicants (Bertram et al., 2019). This finding may be due to the lack of research investigating pesticide impacts on both males and females without considering potential sex differences, an issue seen in other areas of ecotoxicology (Morrison et al., 2024). However, it is important to maintain environmentally relevant sex ratios in exposure experiments to accurately estimate the impacts of pesticide exposure on wild fish (Ford et al., 2021). Similarly, we did not find differences between wild-caught and laboratory-bred fish despite differences being described for other toxicants (Zuberi et al., 2011). This finding may be due to limited research on wild-caught fish. In this regard, we emphasise the importance of studying the impacts of pesticides on wild-caught fish to accurately represent the genetic diversity, physiology and behaviour of wild fish populations (Ford et al., 2021; Zuberi et al., 2011).

Research limitations and future opportunities

While we provide an in-depth synthesis of the impacts of pesticides on fish-conspecific interactions, we must acknowledge several limitations in the literature and our study that offer avenues for future research. We found poor reporting of important methodological items such as the sex of fish, their source, and the behavioural assays used (Figure s3, 4,8). Furthermore, the reporting of data elements, such as raw data, code, and sample sizes, was poor. Consequently, we had to extract data primarily from figures, which may introduce small sources of human error. In some cases, sample sizes had to be assumed based on either the lowest value in a range or the number of data points on a graph. We, therefore, echo calls for better reporting of important methodological items (Hitchcock et al., 2018; Morrison et al., 2024; Ricolfi et al., 2024) and support the development of reporting guidelines such as EthoCRED (Bertram et al., 2024).

The current evidence base has various gaps that limit the breadth of current understanding and provide opportunities for future research. First, we identified four types of conspecific interactions that have been studied in the pesticide literature to date, namely, courtship, aggression, collective movement and social attraction. However, there still remain many other ecologically important social behaviours that are yet to receive attention in the context of pesticide exposures, such as parental care and cooperative behaviours (Goldberg et al., 2020). Second, there has been a wide range of pesticides investigated in the evidence base but research on some modern pesticides such as the neonicotinoids remains limited (Chung et al., 2023; Liu et al., 2023; J. Yang et al., 2023). Likewise, we found limited studies investigating the impacts of pesticide mixtures on fish-conspecific interactions (Hawkey et al., 2021). Third, as previously discussed, most of the research is conducted on zebrafish and many other fish taxa remain largely neglected. Each of the identified gaps provide fruitful scope for future primary research on a wider range of conspecific interactions, pesticides and fish species (Bertram et al., 2022).

Conclusions and boarder implications

In this study, we synthesised the impacts of pesticide exposure on conspecific interactions in fish. Our findings reveal that pesticides generally decrease conspecific social interactions and, most concerningly, reduce courtship behaviours in fish. This reduction in courtship behaviour underscores the importance of considering social contexts in ecotoxicology, as sublethal impacts can impact the likelihood of exposed individuals, successfully attracting mates. Beyond our synthesis findings, we identify key gaps in the existing evidence base and suggest areas for improvement within the literature, noting apparent weaknesses in reporting important methodological details and statistics. Collectively, our findings and the highlighted limitations offer direction for policymakers and researchers on the impacts of pesticide exposure.

Authorship contribution statement

Kyle Morrison: Conceptualisation, Methodology, Software, Formal analysis, Data curation, Writing – Original draft preparation, Writing – Review and Editing; **Gabriel Melhado:** Conceptualisation, Data Curation, Writing – Review and Editing; **Aneesh**

Bose: Conceptualisation, Data Curation, Writing – Review and Editing; **Rhiannon Eastment:** Conceptualisation, Data Curation, Writing – Review and Editing; **Malgorzata Lagisz:** Conceptualisation, Data Curation, Writing – Review and Editing; **Jack L. Manera:** Conceptualisation, Data Curation, Writing – Review and Editing; **Marcus Michelangeli**: Conceptualisation, Data Curation, Writing – Review and Editing; **Shiho Ozeki:** Conceptualisation, Data Curation, Writing – Review and Editing; **Bob B.M. Wong:** Conceptualisation, Writing – Review and Editing; **Yefeng Yang:** Writing – review & editing, Supervision, Software, Methodology, Formal analysis**; Shinichi Nakagawa:** Writing – review & editing, Supervision, Software, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

In preparing this work, the authors used GPT-4o and GPT-4o1-preview by OpenAI, to improve clarity, readability, and writing flow of human-written drafts. Generative AI was also utilized to assist in code annotation. Following the use of these tools, the authors reviewed and edited the content as necessary and take full responsibility for the final publication.

Declaration of competing interest

We declare no conflict of interest.

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Supplementary File 1

Title of submitted paper:

The impacts of pesticide exposure fish conspecific interactions: A systematic review and meta-analysis

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Executive summary of reporting items in the current study:

- PRISMA-EcoEvo checklist includes 88 reporting items, covering 27 reporting domains. Four items were not applicable to our study. Two items were not reported in our study, and the reasons for not reporting them were justified in the *Notes* column.
- Title and abstract (5 YES / 1 NO / 6 items), Aims and questions (5 YES / 5 items), Review registration (3 YES / 3 items), Eligibility criteria (2 YES /2 items), Finding studies (4 YES / 4 items), Study selection (2 YES / 2 items), Data collection process (6 YES / 6 items), Data items (4 YES / 4 items), Assessment of individual study quality (2 YES / 2 items), Effect size measures (2 YES / 1 NA / 3 items), Missing data (2 YES / 2 items), Meta-analytic model description (1 YES / 1 NA / 2 items), Software (5 YES / 5 items), Non-independence (3 YES/ 3 items), Meta-regression and model selection (2 YES / 1 NO / 3 items), Publication bias and sensitivity analyses (3 YES / 3 items), Clarification of post hoc analyses (1 NA / 1 item), Metadata, data, and code (4 YES / 4 items), Results of study selection process (4 YES / 4 items), Sample sizes and study characteristics (4 YES / 4 items), Meta-analysis (1 YES / 1 item), Heterogeneity (1 YES / 1 item), Meta-regression (1 NA / 4 items), Outcomes of publication bias and sensitivity analyses (2 YES / 2 items), Discussion (6 YES / 6 items), Contributions and funding (4 YES / 4 items), References (1 YES / 1 No / 2 items).

Checklist item	Sub-item number	Sub-item	Reported by authors?	Notes
Title and abstract	1.1	Identify the review as a systematic review, meta-analysis, or both	YES	Title section
	1.2	Summarise the aims and scope of the review	YES	Abstract section
	1.3	Describe the data set	YES	Abstract section

	1.4	State the results of the primary outcome	YES	Abstract section
	1.5	State conclusions	YES	Abstract section
	1.6	State limitations	NO	Limitations were stated in *Research limitations and future opportunities* rather than in the Abstract.
Aims and questions	2.1	Provide a rationale for the review	YES	*Introduction*
	2.2	Reference any previous reviews or meta-analyses on the topic	YES	*Introduction*
	2.3	State the aims and scope of the review (including its generality)	YES	*Introduction*
	2.4	State the primary questions the review addresses (e.g. which moderators were tested)	YES	*Introduction*
	2.5	Describe whether effect sizes were derived from experimental and/or observational comparisons	YES	*Methods: Data extraction*
Review registration	3.1	Register review aims, hypotheses (if applicable), and methods in a time-stamped and publicly accessible archive and provide a link to the registration in the methods section of the manuscript. Ideally registration occurs before the search, but it can be done at any stage before data analysis.	YES	*Methods; Open Science Framework: 10.17605/OSF.IO/HDJPQ*
	3.2	Describe deviations from the registered aims and methods	YES	*Methods: Deviations from preregistration*
	3.3	Justify deviations from the registered aims and methods	YES	*Methods: Deviations from preregistration*
Eligibility criteria	4.1	Report the specific criteria used for including or excluding studies when screening titles and/or abstracts, and full texts, according to the aims of the systematic review (e.g. study design, taxa, data availability)	YES	*Methods: Data extraction; Supplementary File 2, Figure s1*
	4.2	Justify criteria, if necessary (i.e. not obvious from aims and scope)	YES	*Methods: Data extraction; Supplementary File 1, Figure s1*

Checklist item	**Sub-item number**	**Sub-item**	**Reported by authors?**	**Notes**

Finding studies	5.1	Define the type of search (e.g. comprehensive search, representative sample)	YES	*Literature screening strategy; Supplementary File 2*
	5.2	State what sources of information were sought (e.g. published and unpublished studies, personal communications)	YES	*Literature screening strategy; Supplementary File 2*
	5.3	Include, for each database searched, the exact search strings used, with keyword combinations and Boolean operators	YES	*Literature screening strategy; Supplementary File 2*
	5.4	Provide enough information to repeat the equivalent search (if possible), including the timespan covered (start and end dates)	YES	*Literature screening strategy; Supplementary File 2*
Study selection	6.1	Describe how studies were selected for inclusion at each stage of the screening process (e.g. use of decision trees, screening software)	YES	*Literature screening strategy; Supplementary File 2*
	6.2	Report the number of people involved and how they contributed (e.g. independent parallel screening)	YES	*Literature screening strategy; Supplementary File 2*
Data collection process	7.1	Describe where in the reports data were collected from (e.g. text or figures)	YES	*GitHub repository*
	7.2	Describe how data were collected (e.g. software used to digitize figures, external data sources)	YES	*Methods: Data extraction*
	7.3	Describe moderator variables that were constructed from collected data (e.g. number of generations calculated from years and average generation time)	YES	*Methods: Data extraction*
	7.4	Report how missing or ambiguous information was dealt with during data collection (e.g. authors of original studies were contacted for missing descriptive statistics, and/or effect sizes were calculated from test statistics)	YES	*Methods: Publication bias, time lag bias and sensitivity analysis*
	7.5	Report who collected data	YES	*Methods: Data extraction*
	7.6	State the number of extractions that were checked for accuracy by co-authors	YES	*Methods: Data extraction*

Checklist item	Sub-item number	Sub-item	Reported by authors?	Notes
Data items	8.1	Describe the key data sought from each study	YES	*Supplementary material 2, Table s3*
	8.2	Describe items that do not appear in the main results, or which could not be extracted due to insufficient information	YES	*Provided in the preregistration: 10.17605/OSF.IO/HDJPQ*
	8.3	Describe main assumptions or simplifications that were made (e.g. categorising both 'length' and 'mass' as 'morphology')	YES	*Supplementary File 2*
	8.4	Describe the type of replication unit (e.g. individuals, broods, study sites)	YES	*Supplementary File 2*
Assessment of individual study quality	9.1	Describe whether the quality of studies included in the systematic review or meta-analysis was assessed (e.g. blinded data collection, reporting quality, experimental *versus* observational)	YES	*Methods: Publication bias, time lag bias and sensitivity analysis*
	9.2	Describe how information about study quality was incorporated into analyses (e.g. meta-regression and/or sensitivity analysis)	YES	*Methods: Publication bias, time lag bias and sensitivity analysis*
Effect size measures	10.1	Describe effect size(s) used	YES	*Methods: Effect size calculations*
	10.2	Provide a reference to the equation of each calculated effect size (e.g. standardised mean difference, log response ratio) and (if applicable) its sampling variance	YES	*Methods: Effect size calculations*
	10.3	If no reference exists, derive the equations for each effect size and state the assumed sampling distribution(s)	NA	*No equations were derived*
Missing data	11.1	Describe any steps taken to deal with missing data during analysis (e.g. imputation, complete case, subset analysis)	YES	*Methods: Statistical modelling summary*
	11.2	Justify the decisions made to deal with missing data	YES	*Methods: Statistical modelling summary*

Checklist item	Sub-item number	Sub-item	Reported by authors?	Notes
	12.1	Describe the models used for synthesis of effect sizes	YES	*Statistical modelling summary; Supplementary File 2*
Meta-analytic model description	12.2	The most common approach in ecology and evolution will be a random-effects model, often with a hierarchical/multilevel structure. If other types of models are chosen (e.g. common/fixed effects model, unweighted model), provide justification for this choice	NA	

Checklist item	Sub-item number	Sub-item	Reported by authors?	Notes
Software	13.1	Describe the statistical platform used for inference (e.g. *R*)	YES	*Methods: Statistical analysis software*
	13.2	Describe the packages used to run models	YES	*Methods: Statistical analysis software; rmd file in the github repository*
	13.3	Describe the functions used to run models	YES	*Methods: Statistical analysis software; rmd file in the github repository*
	13.4	Describe any arguments that differed from the default settings	YES	*Methods: Statistical analysis software; rmd file in the github repository*
	13.5	Describe the version numbers of all software used	YES	*Methods: Statistical analysis software; rmd file in the github repository*
Non-independence	14.1	Describe the types of non-independence encountered (e.g. phylogenetic, spatial, multiple measurements over time)	YES	*Methods: Statistical modelling summary*
	14.2	Describe how non-independence has been handled	YES	*Methods: Statistical modelling summary*
	14.3	Justify decisions made	YES	*Methods: Statistical modelling summary*
Meta-regression and model selection	15.1	Provide a rationale for the inclusion of moderators (covariates) that were evaluated in meta-regression models	YES	*Introduction; Preregistration: 10.17605/OSF.IO/HDJPQ*
	15.2	Justify the number of parameters estimated in models, in relation to the number of effect sizes and studies (e.g. interaction terms were not included due to insufficient sample sizes)	NO	*The parameters were pre-specified in the registration.*

	15.3	Describe any process of model selection	YES	*Methods: Model selection and multi-modal inference*
Publication bias and sensitivity analyses	16.1	Describe assessments of the risk of bias due to missing results (e.g. publication, time-lag, and taxonomic biases)	YES	*Methods: Publication bias, time lag bias and sensitivity analysis*
	16.2	Describe any steps taken to investigate the effects of such biases (if present)	YES	*Methods: Publication bias, time lag bias and sensitivity analysis*
	16.3	Describe any other analyses of robustness of the results, e.g. due to effect size choice, weighting or analytical model assumptions, inclusion or exclusion of subsets of the data, or the inclusion of alternative moderator variables in meta-regressions	YES	*Methods: Publication bias, time lag bias and sensitivity analysis*
Clarification of *post hoc* analyses	17.1	When hypotheses were formulated after data analysis, this should be acknowledged.	NA	No *post-hoc* analyses were performed
Checklist item	**Sub-item number**	**Sub-item**	**Reported by authors?**	**Notes**
Metadata, data, and code	18.1	Share metadata (i.e. data descriptions)	YES	*Supplementary File 2*
	18.2	Share data required to reproduce the results presented in the manuscript	YES	*Supplementary File 2*
	18.3	Share additional data, including information that was not presented in the manuscript (e.g. raw data used to calculate effect sizes, descriptions of where data were located in papers)	YES	*Supplementary File 2*
	18.4	Share analysis scripts (or, if a software package with graphical user interface (GUI) was used, then describe full model specification and fully specify choices)	YES	*All analysis scripts are in the GitHub repository*
Results of study selection process	19.1	Report the number of studies screened	YES	*Figure 1a*
	19.2	Report the number of studies excluded at each stage of screening	YES	*Figure 1a*

	19.3	Report brief reasons for exclusion from the full text stage	YES	*Supplementary File 1*
	19.4	Present a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)-like flowchart (www.prisma-statement.org).	YES	*Figure 1a*
Sample sizes and study characteristics	20.1	Report the number of studies and effect sizes for data included in meta-analyses	YES	*Results: Summary of literature*
	20.2	Report the number of studies and effect sizes for subsets of data included in meta-regressions	YES	*Results: Summary of literature*
	20.3	Provide a summary of key characteristics for reported outcomes (either in text or figures; e.g. one quarter of effect sizes reported for vertebrates and the rest invertebrates)	YES	*Results: Summary of literature*
	20.4	Provide a summary of limitations of included moderators (e.g. collinearity and overlap between moderators)	YES	*Results: Publication bias, time-lag bias and sensitivity analysis*
	20.5	Provide a summary of characteristics related to individual study quality (risk of bias)	YES	*Results: Publication bias, time-lag bias and sensitivity analysis*

Checklist item	**Sub-item number**	**Sub-item**	**Reported by authors?**	**Notes**
Meta-analysis	21.1	Provide a quantitative synthesis of results across studies, including estimates for the mean effect size, with confidence/credible intervals	YES	*Results; Figures 2-6*
Heterogeneity	22.1	Report indicators of heterogeneity in the estimated effect (e.g. I^2, tau^2 and other variance components)	YES	*Results*
Meta-regression	23.1	Provide estimates of meta-regression slopes (i.e. regression coefficients) and confidence/credible intervals	YES	*Results*

	23.2	Include estimates and confidence/credible intervals for all moderator variables that were assessed (i.e. complete reporting)	YES	*Results*
	23.3	Report interactions, if they were included	NA	*No hypothesis on interactions were tested*
	23.4	Describe outcomes from model selection, if done (e.g. R2 and AIC)	YES	*Results: Model selection and multimodal inference*
Outcomes of publication bias and sensitivity analyses	24.1	Provide results for the assessments of the risks of bias (e.g. Egger's regression, funnel plots)	YES	*Results*
	24.2	Provide results for the robustness of the review's results (e.g. subgroup analyses, meta-regression of study quality, results from alternative methods of analysis, and temporal trends)	YES	*Results*
Discussion	25.1	Summarise the main findings in terms of the magnitude of effect	YES	*Discussion*
	25.2	Summarise the main findings in terms of the precision of effects (e.g. size of confidence intervals, statistical significance)	YES	*Discussion*
	25.3	Summarise the main findings in terms of their heterogeneity	YES	*Discussion*
	25.4	Summarise the main findings in terms of their biological/practical relevance	YES	*Discussion*
	25.5	Compare results with previous reviews on the topic, if available	YES	*Discussion*
	25.6	Consider limitations and their influence on the generality of conclusions, such as gaps in the available evidence (e.g. taxonomic and geographical research biases)	YES	*Discussion: Research limitations and future opportunities*

Checklist item	Sub-item number	Sub-item	Reported by authors?	Notes
Contributions and funding	26.1	Provide names, affiliations, and funding sources of all co-authors	YES	
	26.2	List the contributions of each co-author	YES	*We used Contributor Role Taxonomy Frameworks, such as CRediT and MeRIT*
	26.3	Provide contact details for the corresponding author	YES	

	26.4	Disclose any conflicts of interest	YES	*No competing interests*
References	27.1	Provide a reference list of all studies included in the systematic review or meta-analysis	YES	
	27.2	List included studies as referenced sources (e.g. rather than listing them in a table or supplement)		*Will negotiate with journal*

PRISMA-EcoEvo checklist based on: O'Dea, R.E., Lagisz, M., Jennions, M.D., Koricheva, J., Noble, D.W., Parker, T.H., Gurevitch, J., Page, M.J., Stewart, G., Moher, D. and Nakagawa, S. (2021), Preferred reporting items for systematic reviews and meta-analyses in ecology and evolutionary biology: a PRISMA extension. Biol Rev, 2021, 96(5): 1695-1722. https://doi.org/10.1111/brv.12721.

Supplementary File 2

Table of Contents

Supplementary methodology

Literature search

The full search strategy including all search strings, backwards/forwards citation search papers and benchmark studies can be found below.

Scopus

Date: 01/03/2024

Hits: 1508

Search Query:

TITLE-ABS-KEY ((pesticid* OR insecticid* OR herbicid* OR rodenticid* OR piscicid* OR fungicid* OR molluscid* OR larvicid* OR miticid* OR carbamat* OR organophosphat* OR organochlorin* OR pyrethroid* OR chlorpyrifos OR neonicitinoid* OR deltamethrin OR atrazine OR methomyl OR glyphosate OR fipronil OR diazinon OR permethrin OR tebuconazole OR ddt OR dichlorodiphenyltrichloroethane OR dde OR dichlorodiphenyldichloroethylene OR ddd OR dichlorodiphenyldichloroethane OR endosulfan OR *pyrizole OR imidacloprid OR cypermethrin* OR paraquat OR rotenone OR strobilurin OR bifenthrin OR triadimefon OR propiconazole OR difenoconazole OR acetochlor OR fenvalerate OR rotenon* OR triaz* OR linuron OR diuron OR dieldrin OR roundup OR temprid OR lorsban OR orthene or chaindrite OR delforce OR "delta pro" OR bedlam) AND (*fish OR fish* OR shark OR elasmobrach* OR actinopteryg* OR batoidea OR osteichthyes OR teleost* OR guppy OR guppies OR poecil* OR goby OR gobies OR pomatoschistus OR trout* OR oncorhynchus OR minnow* OR medaka OR oryzias OR cyprin* OR stickleback* OR eel OR gasterosteus OR danio OR gambusia OR carp* OR lepomis OR bass OR dicentrarchus OR bream* OR pagrus OR silverside OR meridia OR carassius OR herring OR clupea OR cod OR gadus OR anthobranchia OR fundulus OR salmo* OR tetraodontidae OR teractenos OR batrachoidae OR characin* OR prochilo* OR rhamia OR siluriformes OR heptaptridae) AND (aggress* OR schooli* OR shoal* OR social* OR affiliat* OR defen* OR contes* OR territorialit* OR court* OR alloparent* OR groom* OR vocal* OR dominance OR subordinate OR submi* OR copulat* OR communication OR recognition OR cannibalism OR infanticide OR recognition OR (alarm W/2 signalling) OR (parental W/2 care) OR (maternal W/2 care) OR (paternal W/2 care) OR mating OR (mate W/2 choice) OR (mate W/2 selection) OR (mate W/2 attract*) OR (collective W/2 behav*) OR (reproductive W/2 behav*) OR (collective W/2 decision) OR (collective w/2 motion) OR (group W/2 behav*) OR (group W/2 motion)))

No language, subject area, or year filters will be applied.

Web of Science – Core Collection

Date: 01/03/2024

Hits: 1,396

Search Query:

TS=((pesticid* OR insecticid* OR herbicid* OR rodenticid* OR piscicid* OR fungicid* OR molluscid* OR larvicid* OR miticid* OR carbamat* OR organophosphat* OR organochlorin* OR pyrethroid* OR chlorpyrifos OR neonicitinoid* OR deltamethrin OR atrazine OR methomyl OR glyphosate OR fipronil OR diazinon OR permethrin OR tebuconazole OR ddt OR dichlorodiphenyltrichloroethane OR dde OR dichlorodiphenyldichloroethylene OR ddd OR dichlorodiphenyldichloroethane OR endosulfan OR *pyrizole OR imidacloprid OR cypermethrin* OR paraquat OR rotenone OR strobilurin OR bifenthrin OR triadimefon OR propiconazole OR difenoconazole OR acetochlor OR fenvalerate OR rotenon* OR triaz* OR linuron OR diuron OR dieldrin OR roundup OR temprid OR lorsban OR orthene or chaindrite OR delforce OR "delta pro" OR bedlam) AND (*fish OR fish* OR shark OR elasmobrach* OR actinopteryg* OR batoidea OR osteichthyes OR teleost* OR guppy OR guppies OR poecil* OR goby OR gobies OR pomatoschistus OR trout* OR oncorhynchus OR minnow* OR medaka OR oryzias OR cyprin* OR stickleback* OR eel OR gasterosteus OR danio OR gambusia OR carp* OR lepomis OR bass OR dicentrarchus OR bream* OR pagrus OR silverside OR meridia OR carassius OR herring OR clupea OR cod OR gadus OR anthobranchia OR fundulus OR salmo* OR tetraodontidae OR teractenos OR batrachoidae OR characin* OR prochilo* OR rhamia OR siluriformes OR heptaptridae) AND (aggress* OR schooli* OR shoal* OR social* OR affiliat* OR defen* OR contes* OR territorialit* OR court* OR alloparent* OR groom* OR vocal* OR dominance OR subordinate OR submi* OR copulat* OR communication OR recognition OR cannibalism OR infanticide OR recognition OR (alarm NEAR/2 signalling) OR (parental NEAR/2 care) OR (maternal NEAR/2 care) OR (paternal NEAR/2 care) OR (mate NEAR/2 choice) OR (mate NEAR/2 selection) OR (mate NEAR/2 attract*) OR (collective NEAR/2 behav*) OR (reproductive NEAR/2 behav*) OR (collective NEAR/2 decision) OR (collective NEAR/2 motion) OR (group NEAR/2 behav*) OR (group NEAR/2 motion)))

PubMed

Date: 19/02/2024

Hits: 545

Search Query:

(pesticid* OR insecticid* OR herbicid* OR rodenticid* OR piscicid* OR fungicid* OR molluscid* OR larvicid* OR miticid* OR carbamat* OR organophosphat* OR organochlorin* OR pyrethroid* OR chlorpyrifos OR neonicitinoid* OR deltamethrin OR atrazine OR methomyl OR glyphosate OR fipronil OR diazinon OR permethrin OR tebuconazole OR ddt OR dichlorodiphenyltrichloroethane OR dde OR dichlorodiphenyldichloroethylene OR ddd OR dichlorodiphenyldichloroethane OR endosulfan OR *pyrizole OR imidacloprid OR cypermethrin* OR paraquat OR rotenone OR strobilurin OR bifenthrin OR triadimefon OR propiconazole OR difenoconazole OR acetochlor OR fenvalerate OR rotenon* OR triaz* OR linuron OR diuron OR dieldrin OR roundup OR temprid OR lorsban OR orthene or chaindrite OR delforce OR "delta pro" OR bedlam) AND (*fish OR fish* OR shark OR elasmobrach* OR actinopteryg* OR batoidea OR osteichthyes OR teleost* OR guppy OR guppies OR poecil* OR goby OR gobies OR pomatoschistus OR trout* OR oncorhynchus OR minnow* OR medaka OR oryzias OR cyprin* OR stickleback* OR eel OR gasterosteus OR danio OR gambusia OR carp* OR lepomis OR bass OR dicentrarchus OR bream* OR pagrus OR silverside OR meridia OR carassius OR herring OR clupea OR cod OR gadus OR anthobranchia OR fundulus OR salmo* OR tetraodontidae OR teractenos OR batrachoidae OR characin* OR prochilo* OR rhamia OR siluriformes OR heptaptridae) AND (aggress* OR schooli*

OR shoal* OR social* OR affiliat* OR defen* OR contes* OR territorialit* OR court* OR alloparent* OR groom* OR vocal* OR dominance OR subordinate OR submi* OR copulat* OR communication OR recognition OR cannibalism OR infanticide OR recognition OR “alarm signalling” OR “parental care” OR “maternal care” OR “paternal care” OR “mate choice” OR “mate selection” OR “mate attract*” OR “collective behav*” OR “reproductive behav*” OR “collective decision” OR “collective motion” OR “group behav*” OR “group motion”)

Filtered for Title/Abstract

Backwards/Forwards citation search

The following key reviews on the topic will be used:

1. Bertram, M.G., Martin, J.M., McCallum, E.S., Alton, L.A., Brand, J.A., Brooks, B.W., Cerveny, D., Fick, J., Ford, A.T., Hellström, G. and Michelangeli, M., 2022. Frontiers in quantifying wildlife behavioural responses to chemical pollution. *Biological Reviews*, *97*(4), pp.1346-1364. https://doi.org/10.1111/brv.12844
2. Michelangeli, M., Martin, J.M., Pinter-Wollman, N., Ioannou, C.C., McCallum, E.S., Bertram, M.G., Brodin, T., 2022. Predicting the impacts of chemical pollutants on animal groups. Trends in Ecology & Evolution 37, 789–802. https://doi.org/10.1016/j.tree.2022.05.009
3. Köhler, H.R. and Triebskorn, R., 2013. Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond?. science, 341(6147), pp.759-765. 10.1126/science.1237591
4. Saaristo, M., Brodin, T., Balshine, S., Bertram, M.G., Brooks, B.W., Ehlman, S.M., McCallum, E.S., Sih, A., Sundin, J., Wong, B.B.M., Arnold, K.E., 2018. Direct and indirect effects of chemical contaminants on the behaviour, ecology and evolution of wildlife. Proc. R. Soc. B. 285, 20181297. https://doi.org/10.1098/rspb.2018.1297
5. Shuman-Goodier, M.E., Propper, C.R., 2016. A meta-analysis synthesizing the effects of pesticides on swim speed and activity of aquatic vertebrates. Science of The Total Environment 565, 758–766. https://doi.org/10.1016/j.scitotenv.2016.04.205

6. Söffker, M., Tyler, C.R., 2012. Endocrine disrupting chemicals and sexual behaviors in fish – a critical review on effects and possible consequences. Critical Reviews in Toxicology 42, 653–668. https://doi.org/10.3109/10408444.2012.692114

Grey literature search

Bielefield Academic Search Engine (BASE):

Date: 01/03/2024

Hits: 184

Search Query:

fish* AND behav* AND pesticid* doctype:(14 18*)

ProQuest:

Date: 03/01/2024

Hits: 17

Document type: Dissertation and Thesis

Search Query:

noft(pesticide) AND noft(behavior or behaviour) AND noft(fish)

Benchmark articles

The following benchmark papers will used to test search sensitivity:

1. Boscolo, C.N.P., Pereira, T.S.B., Batalhão, I.G., Dourado, P.L.R., Schlenk, D., de Almeida, E.A., 2018. Diuron metabolites act as endocrine disruptors and alter aggressive behavior in Nile tilapia (Oreochromis niloticus). Chemosphere 191, 832–838. https://doi.org/10.1016/j.chemosphere.2017.10.009
2. Gusso, D., Reolon, G.K., Gonzalez, J.B., Altenhofen, S., Kist, L.W., Bogo, M.R., Bonan, C.D., 2020. Pyriproxyfen Exposure Impairs Cognitive Parameters and Alters Cortisol Levels in Zebrafish. Front. Behav. Neurosci. 14. https://doi.org/10.3389/fnbeh.2020.00103
3. Hawkey, A.B., Glazer, L., Dean, C., Wells, C.N., Odamah, K.-A., Slotkin, T.A., Seidler, F.J., Levin, E.D., 2020. Adult exposure to insecticides causes persistent behavioral and neurochemical alterations in zebrafish. Neurotoxicol. Teratol. 78. https://doi.org/10.1016/j.ntt.2019.106853
4. Jaensson, A., Scott, A.P., Moore, A., Kylin, H., Olsén, K.H., 2007. Effects of a pyrethroid pesticide on endocrine responses to female odours and reproductive behaviour in male parr of brown trout (Salmo trutta L.). Aquatic Toxicol. 81, 1–9. https://doi.org/10.1016/j.aquatox.2006.10.011
5. MacLaren, R.D., 2023. Environmentally Realistic Waterborne Atrazine Exposure Affects Behavior in Poecilia latipinna. Water 15. https://doi.org/10.3390/w15020306
6. Saglio, P., Trijasse, S., 1998. Behavioral responses to atrazine and diuron in goldfish. Arch. Environ. Contam. Toxicol. 35, 484–491. https://doi.org/10.1007/s002449900406
7. Schmidel, A.J., Assmann, K.L., Werlang, C.C., Bertoncello, K.T., Francescon, F., Rambo, C.L., Beltrame, G.M., Calegari, D., Batista, C.B., Blaser, R.E., Roman Júnior, W.A., Conterato, G.M.M., Piato, A.L., Zanatta, L., Magro, J.D., Rosemberg, D.B., 2014. Subchronic atrazine exposure changes defensive behaviour profile and disrupts brain acetylcholinesterase activity of zebrafish. Neurotoxicol. Teratol. 44, 62–69. https://doi.org/10.1016/j.ntt.2014.05.006
8. Shenoy, K., 2012. Environmentally realistic exposure to the herbicide atrazine alters some sexually selected traits in male guppies. PLoS ONE 7. https://doi.org/10.1371/journal.pone.0030611
9. Zaluski, A.B., Wiprich, M.T., de Almeida, L.F., de Azevedo, A.P., Bonan, C.D., Vianna, M.R.M., 2022. Atrazine and Diuron Effects on Survival, Embryo Development, and Behavior in Larvae and Adult Zebrafish. Front. Pharmacol. 13. https://doi.org/10.3389/fphar.2022.841826
10. Zhou, Y., Han, X., Bao, Y., Zhu, Z., Huang, J., Yang, C., He, C., Zuo, Z., 2021. Chronic exposure to environmentally realistic levels of diuron impacts the behaviour of adult marine medaka (Oryzias melastigma). Aquat. Toxicol. 238. https://doi.org/10.1016/j.aquatox.2021.105917

The following string was conducted on Scopus and was used to search for the pre-defined benchmark studies:

TITLE-ABS-KEY ((pesticid* OR insecticid* OR herbicid* OR rodenticid* OR bactericid* OR piscicide OR fungicid* OR molluscid* OR larvicid* OR miticid* OR carbamat* OR organophosphat* OR organochlorin* OR pyrethroid* OR chlorpyrif* OR neonicitinoid* OR deltamethrin OR atrazine OR methomyl OR glyphosate OR fipronil OR diazinon OR permethrin OR tebuconazole OR ddt OR dichlorodiphenyltrichloroethane OR dde OR dichlorodiphenyldichloroethylene OR ddd OR dichlorodiphenyldichloroethane OR endosulfan OR pyrizole OR imidacloprid OR cypermethrin* OR propiconazole OR paraquat OR rotenone OR strobilurin OR bifenthrin OR triadimefon OR propiconazole OR difenoconazole OR acetochlor OR fenvalerate OR phenylpyrzol* OR rotenon* OR triaz* OR linuron OR atrazine* OR diuron OR dieldrin) AND (*fish OR fish* OR shark OR elasmobrach* OR actinopteryg* OR batoidea OR osteichthyes OR teleost* OR guppy OR guppies OR poecilia OR goby OR gobies OR palatoschisis OR trout* OR oncorhynchus OR minnow* OR medaka OR oryzias OR timescales OR cyprin* OR stickleback* OR gasterosteus OR medaka OR oryzias OR danio OR gambusia OR carp* OR cyprinus OR lepomis OR bass OR dicentrarchus OR bream* OR pagrus OR silverside OR meridia OR carassius OR herring OR clupea OR cod OR gadus OR anthobranchia OR fundulus OR salmo* OR tetraodontidae OR teractenos OR batrachoidae) AND (aggress* OR schooli* OR shoal* OR social* OR affiliat* OR defen* OR contes* OR territorialit* OR courtship* OR alloparent* OR groom* OR vocal* OR dominance OR subordinate OR submi* OR copulat* OR communication OR recognition OR cannibalism OR infanticide OR recognition OR (alarm W/2 signalling) OR (parental W/2 care) OR (maternal W/2 care) OR (paternal W/2 care) OR mating OR (mate W/2 choice) OR (mate W/2 selection) OR (mate W/2 attract*) OR (collective W/2 behav*) OR (reproductive W/2 behav*) OR (collective W/2 decision))) AND DOI ("10.1016/j.chemosphere.2017.10.009") OR DOI ("10.1016/j.ntt.2019.106853 ") OR DOI ("10.1016/j.aquatox.2006.10.011 ") OR DOI ("10.3390/w15020306 ") OR DOI ("10.1007/s002449900406 ") OR DOI ("10.1016/j.ntt.2014.05.006 ") OR DOI ("10.3389/fphar.2022.841826 ") OR DOI ("10.1016/j.aquatox.2021.105917 ") OR DOI ("10.3389/fnbeh.2020.00103 ") OR DOI ("10.1371/journal.pone.0030611")

The string successfully found all 10 benchmark studies.

Literature screening strategy

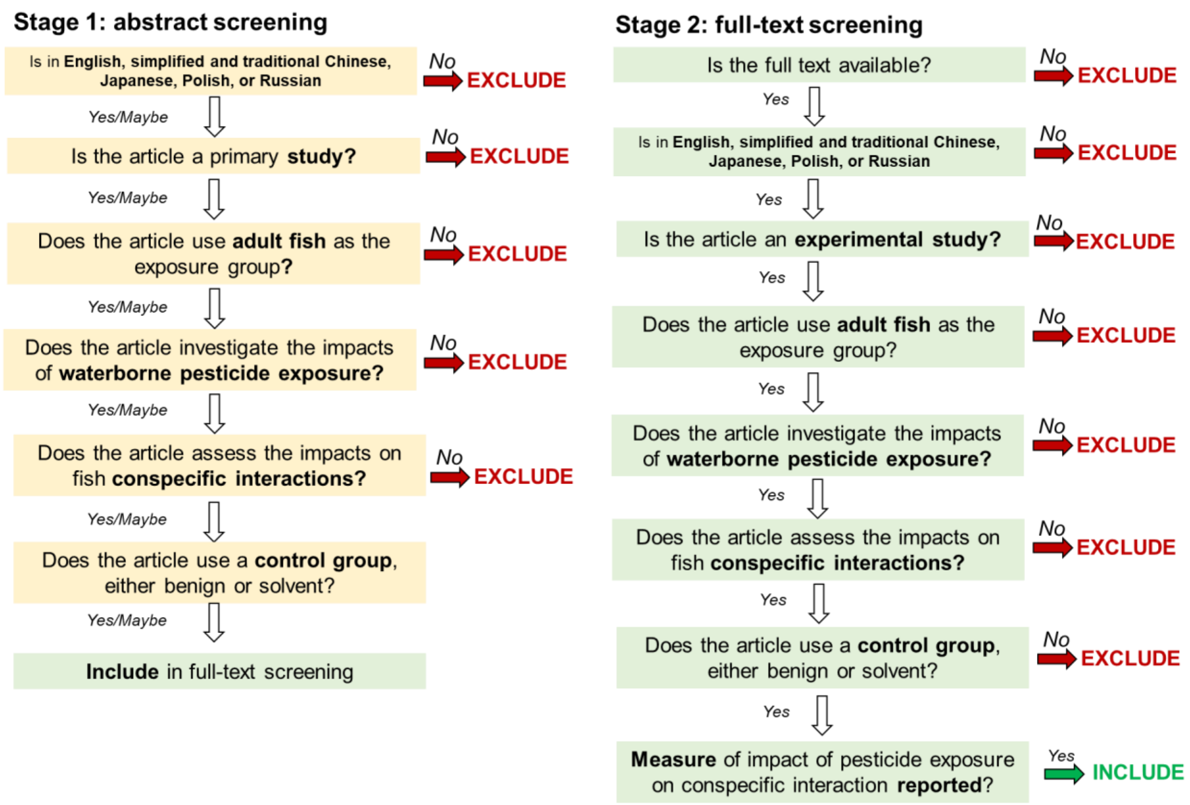

Figure s1) Screening flowchart (decision tree) used for title, abstract and keyword, then full text literature screening

Screening Flowchart additional notes

If the study states that a conspecific interactive behaviour is measured in response to pesticide exposure but does not state that it is a conspecific interaction within the title, abstract or keywords, select “maybe” as a screening decision, with the record being passed to the next round of screening alongside all “yes” decisions for screening. If the behaviour remains ambiguous at full text, then select “reject” decision for screening.

If the study does not state the route of exposure within the title, abstract, or keywords, select “maybe” as a screening decision, with the record being passed to the next round of screening alongside all “yes” decisions. If the route of exposure remains unknown at full-text assessment stage, then select “reject” decision for screening.

If a study within the title, abstract or keywords explicitly states that a study investigates developmental exposure, or the life stage of exposure is not adult (i.e., embryo, larvae or juvenile) reject the study. If the life stage remains unknown during the abstract screening state “maybe”. However, if remains ambiguous during full-text screening reject the study.

Table s1 - Scope of our study according to our predefined PECOST framework

PECOST elements	**Inclusion details**
Population	Studies examine the effects of pesticide exposure on conspecific interactions in fish. We include research that assesses conspecific interactions exclusively in adult fish, with no restrictions on the sex or origin of the fish used.

Exposure	Studies that investigate the effects of pesticides on conspecific interactions in fish, with no limitations on the type or target class of pesticides used. We include studies that assess the effects of exposure at any life stage, provided that conspecific interactions are measured during the adult stage. Examples of chemical classes include organochlorines, organophosphates, carbamates, and neonicotinoids, while examples of target classes include insecticides, rodenticides, herbicides, and fungicides. These lists are not exhaustive, as other chemical and target classes of pesticides exist and will be included. For further reference, consult chemical databases such as the CAS Guide (see https://commonchemistry.cas.org/).
Comparator	The comparator group should involve fish that have not been exposed to pesticides.
Outcome	Experimental studies investigating the impacts of pesticide exposure on non-larval fish conspecific interactions. We define social behaviours broadly as all collective behaviours (e.g., group shoaling, flocking, foraging and collective decision making), social reproductive behaviours (e.g., courtship, mating, and parental care) and animal contests (e.g., aggressions, and territoriality).
Study Type	Experimental studies under a controlled setting which investigate the impacts of pesticide exposure on adult fish social behaviours. Exposure of the pesticide must be during the adult life stage of the fish.
Time Frame	There is no restriction on the publication or conduct date to be included in the meta-analysis.
Additional restrictions	We will exclude full texts that are not published in English, Russian, Polish, Japanese, French, Italian, Simplified Chinese and, Traditional Chinese. We will also exclude studies where data cannot be extracted or there is insufficient data to calculate effect sizes. We will exclude all studies which only report the behavioural change as a proportion or percentage. We will attempt to contact the authors in which data cannot be extracted from studies published later than 01/01/2018.

Table s2 – Studies rejected during full text screening

Study title	**Study DOI**	**Reason for rejection**
Movement analysis of medaka (oryzias latipes) for an insecticide using decision tree	10.1007/11563983_14	wrong outcome
Butyl benzyl phthalate affects shoaling behavior and bottom-dwelling behavior in threespine stickleback	10.1006/enrs.2002.4360	wrong exposure

Responses of the medaka HPG axis PCR array and reproduction to prochloraz and ketoconazole	10.1021/es800591t	wrong outcome
Acute toxicity of an organophosphorus insecticide monocrotophos and its effects on behaviour of an air-breathing fish, Anabas testudineus (Bloch)	not available	full text not available
A study of neurotoxicity of BHC in relation to residual accumulation on the brain tissue of heteropneustes fossilis (Bloch)	not available	full text not available
Antiandrogenic pesticides disrupt sexual characteristics in the adult male guppy (Poecilia reticula)	10.1289/ehp.011091063	wrong exposure
Alteration in sperm release from zebrafish (Brachydanio rerio) exposed to DDT	10.1248/jhs.48.404	wrong outcome
Effects of sublethal concentrations of monocrotophos on the ethological responses of an air-breathing fish, Anabas testudineus (Bloch)	not available	full text not available
Behavior of sunfish exposed to herbicides: A field study	10.1002/etc.5620111011	wrong study type
Response of rainbow trout to a two month exposure to VisionÂ®, a glyphosate herbicide	10.1007/BF00196001	wrong outcome
Pyrethroid induced toxicity to phosphatases in Clarias batrachus (Linn.)	not available	full text not available
Residue studies with [14C] fosamine ammonium in channel catfish	10.1080/15287397909529804	wrong outcome
Joint action of mixtures of toxicants on aquatic organisms	10.1016/0147-6513(82)90020-3	wrong outcome
Multigenerational effects of a complex urban contaminant mixture on the behavior of larval and adult fish in multiple fitness contexts	10.1016/j.scitotenv.2021.148095	wrong population
Effects of a pesticide and a parasite on neurological, endocrine, and behavioral responses of an estuarine fish	10.1016/j.aquatox.2015.09.010	wrong outcome
Behavioral dysfunctions correlate to altered physiology in rainbow trout (<i>Oncorynchus mykiss</i>) exposed to cholinesterase-inhibiting chemicals	10.1007/s002440010149	wrong outcome
Effects of carbofuran on the sea bass (<i>Dicentrarchus labrax</i> L.): Study of biomarkers and behaviour alterations	10.1016/j.ecoenv.2011.07.016	wrong population
The Use of Zebrafish (Danio rerio) Behavioral Responses in Identifying Sublethal Exposures to Deltamethrin	10.3390/ijerph110403650	wrong outcome

Endosulfan exposure inhibits brain AChE activity and impairs swimming performance in adult zebrafish (<i>Danio rerio</i>)	10.1016/j.neuro.2012.03.005	wrong outcome
Changes in behavior and brain acetylcholinesterase activity in mosquito fish, Gambusia affinis in response to the sub-lethal exposure to chlorpyrifos.	10.3390/ijerph2005030013	wrong outcome
Sublethal effects of monocrotophos on locomotor behavior and gill architecture of the mosquito fish, <i>Gambusia affinis</i>	10.1080/03601230500227509	wrong outcome
Acute and subchronic toxic effects of atrazine and chlorpyrifos on common carp (<i>Cyprinus carpio L</i>.): Immunotoxicity assessments	10.1016/j.fsi.2015.04.016	wrong outcome
Aggressive behaviour in <i>Betta splendens</i> as a bio-indicator of freshwater pollution	not available	full text not available
Male mate choice selects for female coloration in a fish	10.1073/pnas.211439298	wrong exposure
Temporal pattern in swimming activity of two fish species (<i>Danio rerio</i> and <i>Leucaspius delineatus</i>) under chemical stress conditions	10.1080/09291010500103112	wrong outcome
Guppy sexual behavior as an effect biomarker of estrogen mimics	10.1006/eesa.1999.1766	wrong exposure
Effects of an endocrine disrupter on courtship and aggressive behaviour of male three-spined stickleback, <i>Gasterosteus aculeatus</i>	10.1006/anbe.2001.1824	wrong exposure
Impairment of the reproductive potential of male fathead minnows by environmentally relevant exposures to 4-nonylphenolf	10.1016/j.aquatox.2007.10.004	data cannot be extracted
Use of behavioral endpoints to determine protective concentrations of the insecticide fonofos for bluegill (Lepomis macrochirus)	10.1007/BF00212556	wrong study type
Chemobehavioral Changes Induced by Short-Term Exposures to Prochloraz, Nicosulfuron, Carbofuran in Goldfish	10.1007/s00244-003-2223-6	wrong population
DDT induced ethological changes in estuarine fish	10.1007/BF00005932	wrong outcome
Schooling behavior of Menidia medidia in the presence of the insecticide Sevin (Carbaryl)	10.1007/BF00388493	wrong population
Chlorpyrifos disrupts social behavior in adult zebrafish	10.1016/j.ntt.2014.04.058	full text not available
DDT CAUSES CHANGES IN ACTIVITY AND SCHOOLING BEHAVIOR IN GOLDFISH	10.1016/0013-9351(74)90076-0	data cannot be extracted
Opercular display in male betta splendens can be used as a bioindicator of disulfoton pollution	not available	full text not available

Gardening behaviour of Sicydium punctatum (Gobioidei: Sicydiinae): in vitro experiments in the context of chlordecone pollution in Guadeloupe Island rivers	not available	wrong outcome
Acute toxicity and effects of the roundup transorb, a glyphosate-based herbicide, on freshwater teleost matrinxÃ£, brycon amazonicus	10.22034/iar.2021.1910474.1099	wrong population
Waterborne agrichemicals compromise the anti-predatory behavior of zebrafish	10.1007/s11356-020-09862-2	wrong outcome
Adhesion Molecule L1 Agonist Mimetics Protect Against the Pesticide Paraquat-Induced Locomotor Deficits and Biochemical Alterations in Zebrafish	10.3389/fnins.2020.00458	wrong population
Cypermethrin Influence on Oxidative Status and Anxious Behaviour in <i>Paracheirodon innensi</i> Species	not available	full text not available
Monocrotophos Based Pesticide Alters the Behavior Response Associated with Oxidative Indices and Transcription of Genes Related to Apoptosis in Adult Zebrafish (Danio rerio) Brain	10.13005/bpj/1998	wrong outcome
Do you smell the danger? Effects of three commonly used pesticides on the olfactory-mediated antipredator response of zebrafish (Danio rerio)	10.1016/j.chemosphere.2019.124963	wrong outcome
Ecological restructuring in experimental aquatic mesocosms due to the application of diflubenzuron	10.1002/etc.5620151023	wrong outcome
Neurobehavioral, physiological and inflammatory impairments in response to bifenthrin intoxication in <i>Oreochromis niloticus</i> fish: Role of dietary supplementation with <i>Petroselinum crispum</i> essential oil	10.1016/j.aquatox.2020.105715	wrong outcome
Effects of sublethal and realistic concentrations of the commercial herbicide atrazine in Pacu (<i>Piaractus mesopotamicus</i>): Long-term exposure and recovery assays	10.14202/vetworld.2020.147-159	wrong population
Pathological Effects and Lethal Concentration of Two Nonionic, Tallowamine-Polyethoxylate Surfactants in White Cachama Piaractus brachypomus	10.1007/s11270-019-4340-5	wrong exposure
Comparisons of tissue-specific transcription of stress response genes with whole animal endpoints of adverse effect in striped bass (<i>Morone saxatilis</i>) following treatment with copper and esfenvalerate	10.1016/j.aquatox.2007.07.011	wrong population

Toxicological impact of pentachlorophenol on the hepatic and reproductive activity of the stinging catfish heteropneustes fossilis	10.4194/2618-6381-v19_2_07	wrong outcome
Physiological and biochemical responses of Nile tilapia (<i>Oreochromis niloticus</i>) to acute trichlorfon exposure	10.22034/IAR.2020.1904943.1071	wrong population
Histological alteration in different tissues of indian major carp, labeo rohita (Hamilton) exposed to profenofos 50% EC and carbosulfan 25% EC formulations	10.15412/J.JBTW.01060301	wrong outcome
Exposure to fenvalerate and tebuconazole exhibits combined acute toxicity in zebrafish and behavioral abnormalities in larvae	10.3389/fenvs.2022.975634	wrong population
Behavioral, biochemical, and endocrine responses of zebrafish to 30-min exposure with environmentally relevant concentrations of imidacloprid-based insecticide	10.1007/s11356-023-27667-x	data cannot be extracted
Sublethal effects of the organic antifoulant MexelÂ®432 on osmoregulation and xenobiotic detoxification in the flatfish Solea senegalensis	10.1016/j.chemosphere.2009.12.054	wrong population
Dietary exposure of largemouth bass to OCPs changes expression of genes important for reproduction	10.1016/j.aquatox.2006.05.003	wrong outcome
Binary mixture of DDT and Arochlor1254: Effects on sperm release by Danio rerio	10.1016/j.ecoenv.2003.11.003	wrong outcome
Acute toxicity and histopathological alterations of RoundupÂ® herbicide on "cachama blanca" (<i>Piaractus brachypomus</i>)	10.1590/S0100-736X2008001100002	wrong population
Glyphosate induces cardiovascular toxicity in <i>Danio rerio</i>	10.1016/j.etap.2016.08.010	wrong outcome
Environmental levels of azoxystrobin disturb male zebrafish behavior: Possible roles of oxidative stress, cholinergic system, and dopaminergic system	10.1016/j.ecoenv.2023.115744	wrong outcome
Rotenone alters behavior and reproductive functions of freshwater catfish, <i>Mystus cavasius</i>, through deficits of dopaminergic neurons in the brain	10.1016/j.chemosphere.2020.128355	wrong outcome
Biochemical and behavioral effects of carbofuran in goldfish (<i>Carassius auratus</i>)	10.1897/1551-5028	wrong population
Sublethal effects of the pesticide Diazinon on olfactory function in mature male Atlantic salmon parr	10.1006/jfbi.1996.0075	wrong outcome
Combined effects of high temperature and pesticide mixture exposure on free-swimming	10.1080/15287394.2023.2174463	wrong outcome

behaviors and hepatic cytochrome P450 1A expression in goldfish, Carassius auratus		
Multistress effects on goldfish (<i>Carassius auratus</i>) behavior and metabolism	10.1007/s11356-015-5147-6	wrong outcome
UNRAVELING MARKOV PROCESSES IN MOVEMENT PATTERNS OF INDICATOR SPECIES IN RESPONSE TO CHEMICAL STRESSORS	10.1142/S0217984911026851	wrong outcome
Acute exposure of embryo, larvae and adults of <i>Danio rerio</i> to fipronil commercial formulation reveals effects on development and motor control	10.1007/s10646-021-02497-0	wrong outcome
Impacts of Endothall Applications on Largemouth Bass Spawning Behavior and Reproductive Success	10.1577/M08-041.1	wrong outcome
METHYL PARATHION REDUCES AGGRESSIVE BEHAVIOUR OF MALE <i>BETTA SPLENDENS</i>	not available	full text not available
The effects of estrogenic pesticide on reproductive behavior of Cyprinodon variegatus, the sheepshead minnow.	10.1002/etc.5620220425	wrong exposure
Long-term exposure to polyethylene microplastics and glyphosate interferes with the behavior, intestinal microbial homeostasis, and metabolites of the common carp (Cyprinus carpio L.)	10.1016/j.scitotenv.2021.152681	wrong population
Behavioral Impairment in Aquatic Organisms Exposed to Neurotoxic Pollutants	10.3390/toxics10050243	wrong study type
The effects on brown trout (<i>Salmo trutta fario</i>) of different concentrations of deltamethrin	10.1016/j.cbpc.2019.108606	wrong outcome
Impact of chlorpyrifos on behavior and histopathological indices in different tissues of freshwater fish Channa punctatus (Bloch)	10.1007/s11356-019-05165-3	wrong outcome
EFFECTS OF SOME INSECTICIDES ON RAINBOW-TROUT DEFENCE MECHANISM	10.1016/j.cbpc.2021.109064	wrong outcome
THE EFFECT OF SUBLETHAL CONCENTRATION OF DECIS 2.5 EC PESTICIDE ON LEARNING AND MEMORY PROCESSES IN COMMON CARP, <i>CYPRINUS CARPIO</i> (ACTINOPTERYGII: CYPRINIFORMES: CYPRINIDAE)	10.3750/AIP2010.40.2.07	wrong outcome
Evaluation of sub-lethal effects of endosulfan on cortisol secretion, glutathione S-transferase and acetylcholinesterase activities in <i>Clarias</i> <i>gariepinus</i>	10.1016/j.fct.2010.10.025	wrong population
Vitamin B₁₂ Ameliorates Pesticide-Induced Sociability Impairment in Zebrafish	10.3390/ani14030405	wrong population

(<i>Danio rerio</i>): A Prospective Controlled Intervention Study		
Reproductive consequences of a changing world: effects of the pesticide bifenthrin on mosquitofish reproductive behavior	10.1016/j.aquatox.2018.12.001	data cannot be extracted
LOW CONCENTRATION EFFECTS OF ENDOSULFAN INSECTICIDE ON REPRODUCTIVE-BEHAVIOR IN THE TROPICAL CICHLID FISH SAROTHERODON-MOSSAMBICUS	10.1007/BF01625587	full text not available
Behavioral and olfactory responses to prochloraz, bentazone, and nicosulfuron-contaminated flows in goldfish.	10.1007/s002440010237	wrong population
NON-LETHAL CONCENTRATION OF PARAOXON IMPAIRS AGGRESSIVE OPERCULAR DISPLAY IN MALE SIAMESE FIGHTING FISH, <i>Betta splendens</i>	not available	full text not available
TOXICITY EFFECT OF CYPERMETHRIN (10% EC) TO THE FRESHWATER FISH <i>CIRRHINUS MRIGALA</i> (HAMILTON)	not available	wrong outcome
Developing a novel quantitative parameter for characterizing spatial distribution of fish following exposure to chemicals and wastewater: Behavioral Gini coefficient.	10.1016/j.jes.2023.06.002	wrong population
Sublethal effects of an organophosphate insecticide on the European eel, Anguilla anguilla.	10.1006/eesa.1996.1488	wrong outcome
Diazinon disrupts antipredator and homing behaviors in chinook salmon (<i>Oncorhynchus tshawytscha</i>)	10.1139/cjfas-57-9-1911	wrong outcome
Developmental, behavioral, and reproductive effects experienced by Japanese medaka (<i>Oryzias latipes</i>) in response to short-term exposure to endosulfan	10.1016/S0147-6513(02)00005-2	wrong population
Pattern recognition of the movement tracks of medaka (<i>Oryzias latipes</i>) in response to sub-lethal treatments of an insecticide by using artificial neural networks	10.1016/S0269-7491(02)00183-5	wrong population
Stage-dependent effects of chlorpyrifos on medaka (<i>Oryzias latipes</i>) swimming behavior using a miniaturized swim flume	10.1016/j.aquatox.2018.04.008	wrong population
Computational analysis of movement behaviors of medaka (<i>Orydas latipes</i>) after the treatments of copper by using fractal dimension and artificial neural networks	10.2495/ETOX060101	wrong exposure
Behavioral effects of waterborne carbofuran in goldfish.	10.1007/BF00212371	wrong population

Behavioural changes in freshwater fish, Cirrhinus mrigala (Hamilton) exposed to cypermethrin.	not available	full text not available
Neurodepressive action of a piscicidal glycoside of plant, Aesculus indica (Colebr.) in fish.	not available	full text not available
Rapid microdetection of organochlorine pesticides in submilligram fish tissue samples.	10.1007/BF01560914	full text not available
Neuroprotective effects of nanogold-based Ayurveda medicine Suvarna Bhasma against rotenone-induced Parkinson's-like model	10.1016/j.jaim.2023.100854	wrong outcome
Impairment of trophic interactions between zebrafish (<i>Danio rerio</i>) and midge larvae (<i>Chironomus riparius</i>) by chlorpyrifos	10.1007/s10646-010-0516-x	wrong outcome
Transcriptomic changes underlie altered egg protein production and reduced fecundity in an estuarine model fish exposed to bifenthrin	10.1016/j.aquatox.2016.02.014	wrong outcome
Effect of methyl parathion and chlorpyrifos on certain biomarkers in various tissues of guppy fish, <i>Poecilia reticulata</i>	10.1016/j.pestbp.2011.09.002	wrong outcome
ALARM SUBSTANCE RECOGNITION AND PREDATOR AVOIDANCE BY CHINOOK SALMON (<i>ONCORHYNCHUS TSCHAWYTSCHA</i>) FOLLOWING EXPOSURE TO AN ORGANOPHOSPHATE PESTICIDE	10.1002/etc.142	wrong outcome
Devicyprin induced gonadal impairment in a freshwater food fish, <i>Channa punctatus</i> (Bloch)	not available	full text not available
Biochemical, haematological and oxidative stress responses of common carp (Cyprinus carpio L.) after sub-chronic exposure to copper	10.17221/8681	wrong exposure
Toxicological effects of a glyphosate-based formulation on the liver of Poecilia reticulata	not available	full text not available
Acute and chronic toxicity of the benzoylurea pesticide, lufenuron, in the fish, <i>Colossoma macropomum</i>	10.1016/j.chemosphere.2016.07.033	no result reported
Changes in reproductive biomarkers in an endangered fish species (bonytail chub, Gila elegans) exposed to low levels of organic wastewater compounds in a controlled experiment	10.1016/j.aquatox.2009.08.008	wrong exposure
Toxicological effects of lambda-cyhalothrin on liver, kidney and testis of indian catfish clarias batrachus	10.22506/ti/2015/v22/i3/137637	wrong outcome
Vision-based real-time monitoring on the behavior of zebrafish school	not available	wrong exposure

Environmentally relevant concentrations of bifenthrin affect the expression of estrogen and glucocorticoid receptors in brains of female western mosquitofish	10.1016/j.aquatox.2018.12.001	data cannot be extracted
Mechanistic revealing of reproductive behavior impairment in male guppy (Poecilia reticulata) induced by environmentally realistic 2,2â€²-dithiobis-pyridine exposure	10.1016/j.chemosphere.2021.131839	wrong exposure
Risky business: Changes in boldness behavior in male Siamese fighting fish, Betta splendens, following exposure to an antiandrogen	10.1016/j.envpol.2018.01.029	data cannot be extracted
Efeitos tÃ³xicos e genotÃ³xicos do herbicida Roundup TransorbÂ® em Guppy (Poecilia reticulata) submetido a tratamento agudo ; Toxic effects and genotoxicity of Roundup TransorbÂ® in Guppy(Poecilia reticulata) submitted to acute treatment	not available	wrong language
Impactos neuroendÃ³crinos e comportamentais de um inseticida Ã base de imidacloprid em zebrafish ; Neuroendocrine and behavioral impacts of an imidacloprid-based insecticide on zebrafish	not available	full text not available
Response on multistress effects on goldfish (carassius auratus) ; RÃ©ponse aux stress multiples chez les poissons : effets croisÃ©s de la tempÃ©rature et des cocktails de pesticides	not available	wrong outcome
Respostas bioquÃmicas e comportamentais de peixe-zebra (Danio rerio) expostos a imidacloprido: avaliaÃ§Ã£o do dano oxidativo e perfil antioxidante ; Biochemical and behavioral responses of zebrafish exposed to imidacloprid: assessment of oxidative damage and antioxidant profile	not available	wrong outcome
Investigation on acute toxicity and behavioral changes in Channa punctatus (Bloch) due to organophosphate pesticide profenofos ; Not Available	10.3109/01480545.2011.585650	wrong outcome
Lethal concentration and toxicity stress of Carbosulfan, Glyphosate and Atrazine to freshwater air breathing fish Channa punctatus (Bloch) ; Not Available	not available	wrong outcome
Fish behavior: A promising model for aquatic toxicology research	10.1016/j.scitotenv.2019.06.028	wrong population
Investigation of acute toxicity and behavioral response of Indian major carp, Cirrhinus mrigala (Hamilton, 1822) in response to Cypermethrin ; Not Available	not available	wrong outcome

Biochemical and physiological indicators of behavioral impairment in salmonids exposed to chlorpyrifos and copper	not available	wrong outcome
Olfactory toxicity in zebrafish : Assessing the effects of three commonly used pesticides and cadmium on the olfactory system of Danio rerio ...	not available	duplicate paper (thesis)
Assessing the effects of MACADAMIA ORCHARD pesticide inputs on recipient aquatic ecosystems	not available	wrong population
Effects of diuron and 3,4-dichloroaniline on reproduction and early development of Javanese medaka (Oryzias javanicus, Bleeker 1854)	not available	wrong outcome
Endocrine disruption in context: dose, compound, and route of exposure interact to affect the multivariate phenotype in mangrove rivulus fish (kryptolebias marmoratus)	10.1111/jfb.12814	wrong exposure
The effects of organophosphate exposure on non target terrestrial and aquatic organisms following different exposure regimes : linking biomarker responses and life-cycle effects	not available	wrong outcome
Toxicidade aguda, comportamento e anÃ¡lises anatomopatolÃ³gicas em diferentes espÃ©cies de peixes expostos Ã formulaÃ§Ã£o comercial contendo deltametrina ; Acute toxicity, behavior and anatomopathological analysis in different species of fish exposed to the commercial formulation containing deltamethrin	not available	full text not available
Neurotoxicity of pesticides to salmon: Physiology to ethology	not available	wrong outcome
Behavioral and Physiological consequences of pesticide exposure for estuarine fishes	10.18311/ti/2023/v30i4/34317	wrong outcome
Anthropogenic Impacts on Freshwater Organisms: Bioassessments from the Molecular to Community Levels	not available	wrong population
Impact, recovery and carryover effect of RoundupÂ® on predator recognition in common spiny loach, Lepidocephalichthys thermalis.	10.1007/s10646-018-02011-z	wrong outcome
Propiconazole induces abnormal behavior and oxidative stress in zebrafish.	10.1007/s11356-019-05977-3	wrong outcome
Behavioral responses to atrazine and diuron in goldfish.	10.1007/s002449900406	wrong population
Neurotoxicity Assessment in Adult Danio rerio using a Battery of Behavioral Tests in a Single Tank.	10.3791/65869	wrong exposure

Evaluation of the acute and sublethal toxicity of Mancozeb in PacamÃ£ (Lophiosilurus alexandri).	10.1590/1519-6984.274393	wrong outcome
Abamectin promotes behavior changes and liver injury in zebrafish.	10.1016/j.chemosphere.2022.136941	wrong outcome

Data Extraction and effect size calculations

Data extraction variables

Table s3) Table of all extracted variables and descriptions

Items	**Description**	**Predefined_options**
comment_checker	Provide any comments necessary regarding data checking	N/A
comment_extractor	Provide any comments necessary regarding data extraction	N/A
initials_checker	Write the initials of the data checker in capital letters (e.g. KM).	N/A
initials_extractor	Write the initials of the data extractor in capital letters (e.g. KM).	N/A
Study identification information		
title	Provide the full title of the eligible study being extracted.	N/A
doi	Provide the short form doi of the eligible study (e.g., 10.32942/X2231N).	N/A
url	Provide the full URL linking to the article (e.g., https://ecoevorxiv.org/repository/view/6169/).	N/A
journal	Provide the full name of the journal or the platform where the data is reported.	N/A
study_id	Provide the year when the study was published.	N/A
exp_id	Use the format "study_name_exp_n" to create a unique identifier of each exposure group within an eligible study (e.g., morrison_2023_exp_001). When the cohort is unclear between behaviours give the same exposure ID.	N/A
exp2_id	Use the format "study_name_exp2_n" to create a unique identifier of each exposure	N/A

	group within an eligible study (e.g., morrison_2023_exp_001). When the cohort is unclear between behaviours give a new exposure ID.	
assay_id	Use the format "study_name_exp_n_assay_n" to create a unique identifier of each exposure within an eligible study (e.g., morrison_2024_exp_001_assay_001). This assay ID is for the exp_ID.	N/A
assay2_id	Use the format "study_name_exp2_n_assay2_n" to create a unique identifier of each exposure within an eligible study (e.g., morrison_2024_exp2_001_assay2_001). This assay ID is for the exp2_ID.	N/A
Population Attributes		
species_english	Provide the common name of species in English as reported in the paper. If the paper does not report common name in English state "not reported".	N/A
species_latin	Provide the current binomial name (i.e., the scientific name) of species investigated in the eligible study.	N/A
source	Provide the source of species used in the eligible study experiment (e.g., wild collected or commercial supplier).	wild collected
		commercial supplier
		laboratory stock from wild population
		laboratory stock from commercial supplier
		not reported
		other
sex	Provide the sex of the exposed fish population (e.g., female). If the population is mixed sex state "mixed".	male
		female
		mixed
		not reported
		other
Pesticide characteristics		
pesticide	Provide the name for the pesticide used in the eligible study. This may be a commercial mixture of pesticides.	N/A
pesticide_grade	Provide the chemical grade of the pesticide used in the eligible study. If the	analytical
		commercial
		not reported

	chemical grade is not reported state "not reported".	
cas_id	Provide the CAS identification code of the chemical as reported in the eligible study. If the CAS identification number is not reported externally source CAS id code via: https://www.cas.org/. If externally sourced specify in “cas_id_comment”	N/A
cas_id_comment	Provide a comment on how the CAS identification code was retrieved. Either from the primary study or external link.	N/A
pesticide_chemical_class	Provide the chemical class of the pesticide used in the eligible study.	N/A
dosage	Provide the dosage of the pesticide used in the eligible study. If the dosage of exposure is not reported state "not reported".	N/A
dosage_unit	Provide the unit of the dosage of the pesticide used in the eligible study. If the dosage of exposure is not reported state "not reported".	pg/L
		ng/L
		ug/L
		mg/L
		g/L
		not reported
		other
duration	Provide the duration of the pesticide exposure used in the eligible study. If the duration is not reported state "not reported".	N/A
duration_unit	Provide the unit of the duration of pesticide exposure used in the eligible study. If the duration is not reported state "not reported".	seconds
		minutes
		hours
		days
		weeks
		months
		not reported
		other
control_solvent	Provide the solvent used within the control group. If there is none state “none” and if the solvent is not reported state “not reported”.	N/A
temperature	Provide the temperature of the water during the pesticide exposure.	N/A
temperature_unit	Provide the unit of the temperature of water during pesticide exposure. If the temperature is not reported state “not reported”.	

Behaviour Attributes		
behaviour_measured	Provide the behaviour being assessed in the study.	N/A
behaviour_measured_standardised	Provide the behaviour being assessed using standardised terminology. This will be developed after extraction.	Aggression Collective arrangement Courtship Social Attraction
behaviour_assay	Provide the assay being used to assess the behaviour. Use the wording as described in the study.	N/A
behavioural_assay_standardised	Provide the assay being used to assess the behaviour using standardised terminology.	N/A
behavioural_assay_standardised2	Provide the whether the assay being used is zonal or a behaviour count. This variable is only recorded for aggression and sociality.	zonal count entries
Statistics		
data_source	Provide the location of where the extracted data was reported such as text page (e.g.,text_p4), figure (e.g., fig2), table (e.g., table3), or supplementary material (e.g.,supplement_fig2).	N/A
data_file	If the data was reported in a table or figure, please screenshot the source and save in the following format author_year_fign or author_year_tablen (n being the figure or table number). If the data is not reported in a figure or table state "N/A".	N/A
control_n	Provide the sample size of the control group (i.e., number of unmanipulated individuals measured for the given outcome).	N/A
control_mean	Provide the mean of the measured outcome value for the control group (i.e., unmanipulated individuals).	N/A
control_sd	Provide the standard deviation of the measured outcome value for the (i.e., unmanipulated individuals). If measurement uncertainties were reported as other forms such as confidence interval, convert it to SD or SE (see below) and take notes in the "statistic_comment_control" column.	N/A

control_se	Provide the standard error of the measured outcome value for the (i.e., unmanipulated group). If measurement uncertainties were reported as other forms such as confidence interval, convert it to SD or SE and take notes in the "statistics_comment_control" column.	N/A
statistics_comment_control	Provide a note regarding the control statistics such as any calculations needed.	N/A
treatment_n	Provide the sample size of treatment group (i.e., number of individuals in the exposed group for the given outcome)	N/A
treatment_mean	Provide the mean of the measured outcome value for the treatment group (i.e., exposed group)	N/A
treatment_sd	Provide the standard deviation of the measured outcome value for the treatment group (i.e., exposed group). If measurement uncertainties were reported as other forms such as confidence interval, convert it to SD or SE (see below) and take notes in the "statistics_comments_treatment" column.	N/A
treatment_se	Provide the standard error of the measured outcome value for the treatment group (i.e., exposed group). If measurement uncertainties were reported as other forms such as confidence interval, convert it to SD or SE (see below) and take notes in the "statistics_comments_treatment" column.	N/A
statistics_comment_treatment	Provide notes regarding the control statistics such as any calculations needed.	N/A

Data extraction additional notes

To analyse the behaviours extracted from each study, we standardised the measured behaviours into the following categories:

1) Behaviours related to fish aggression, such as the number of bites, time spent in the aggression zone, or entries into the aggression zone, were categorized as “aggression.”
2) Behaviours related to mating, such as time spent near the opposite sex, mating attempts, or distance to a female (for males), were categorized as “courtship.”
3) Behaviours reflecting social interactions between two individuals, such as time near a mirror, time near a conspecific, or time spent in a social zone, were categorized as “social attraction.”
4) Behaviours involving social interactions among multiple individuals, such as shoaling speed, shoal size, and shoal arrangement, were categorized as “collective arrangement”

No other behaviours were observed in the current literature base.

To analyse the behavioural assays extracted from each study, we standardised the assays used into the following categories:

Model parameters

Intercept-only model: Examine the overall effect of the impacts pesticide exposure on conspecific interactions mean and variability

We constructed an intercept only model for both lnRR and lnVR to estimate the overall impacts of pesticide exposure on conspecific interactions:

$$ES_{[i]} = \beta_0 + u_{study[j]} + u_{observation[j]} + u_{pesticide[j]} + u_{species[j]} + e_{[j]}$$

where $ES_{[i]}$ is lnRR or lnVR, β_0 is the intercept; $u_{study[j]}$ is the study-level random effect of the jth study accounting for multiple effect sizes from a single study; $u_{observation[j]}$ is the observation-level random effect capturing observation or residual variance; $u_{pesticide[j]}$ is the pesticide specific random effect for the jth pesticide accounting for correlations in effect size estimates from the same pesticide; $u_{species[j]}$ is the species specific random effect accounting for correlations in effect size estimates from the same species; $e_{[j]}$ is the sampling variance-covariance effect which accounts for the precision of the effect size estimate and correlation between sampling errors. The term $e_{[j]}$ follows a multivariate normal distribution with the mean equal to 0 and variance–covariance $\mathbf{V}$ where the diagonal elements represent the sampling variance of the effect size estimate, and the off-diagonal elements represent the sampling covariance with an assumed constant variance of $\rho \quad = 0.5$. The $u_{phylogeny[j]}$ did not contribute any variation to the model and thus was not included in meta-regression models. We also did not implement robust variance estimation as it did not provide a more accurate estimate of $\mathbf{V}$. All the random effects follow a normal distribution with a mean 0 and variance component $N(0, \sigma^2 \mathbf{I})$, where σ^2 denotes either $\sigma^2{}_{study}$, $\sigma^2{}_{oberservation}$, $\sigma^2{}_{pesicide}$ or $\sigma^2{}_{species}$ estimated from the model REML, $\mathbf{I}$ = identity matrix.

Predictor models: Examining the moderating factors for the impacts pesticide exposure on conspecific interactions mean and variability

To assess whether effect size estimates were influenced by predefined predictor variables (see supplementary file x, section x for all predictor variables).

$$ES_{[i]} = \beta_0 + \beta\boldsymbol{X} + u_{study[j]} + u_{observation[j]} + u_{pesticide[j]} + u_{species[j]} + e_{[j]}$$

Where β_0 is a vector of slopes for each level of the moderator variable and βX is a design matrix of the moderator variables. All other notations are defined in section "Intercept only model".

Supplementary results

Summary of literature

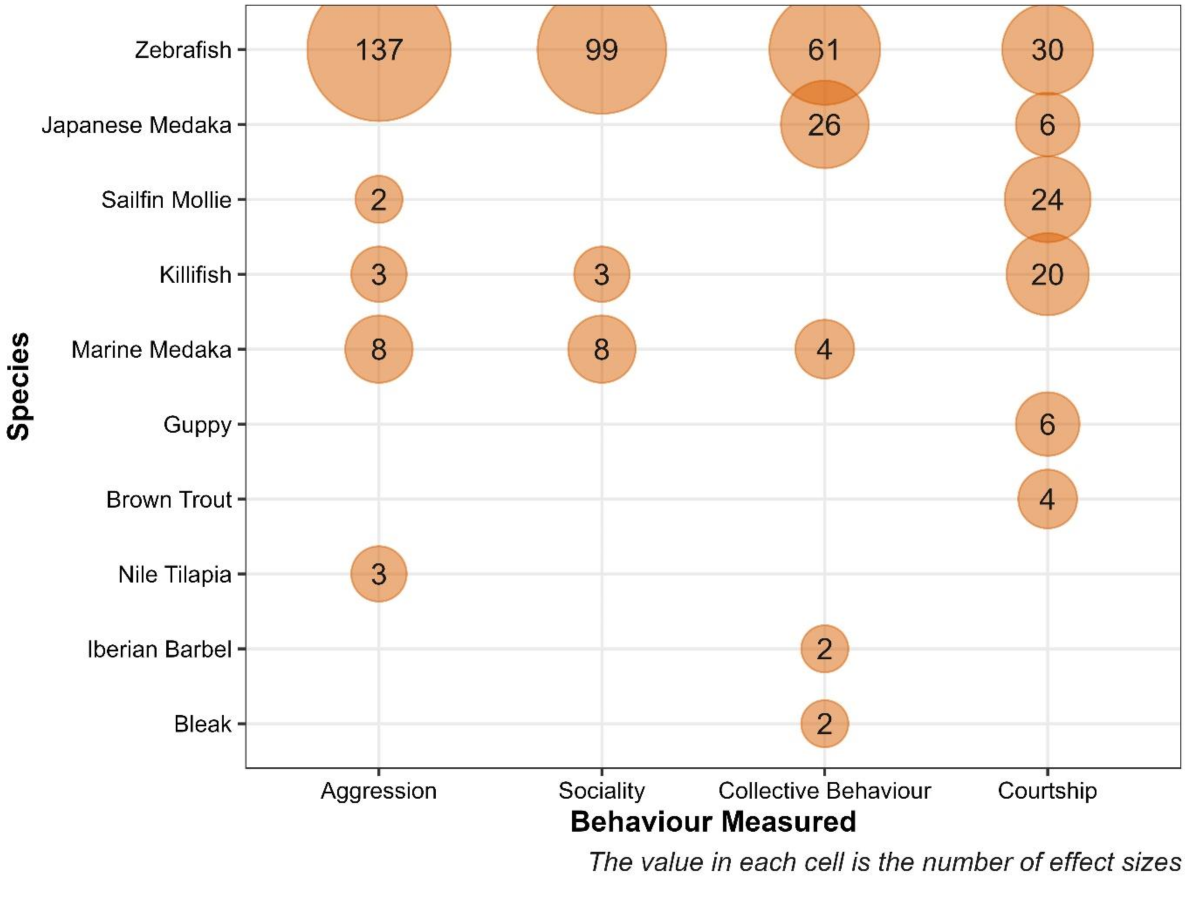

Figure s2) A bubble plot showing the number of effect sizes of each species per conspecific interaction behaviour described

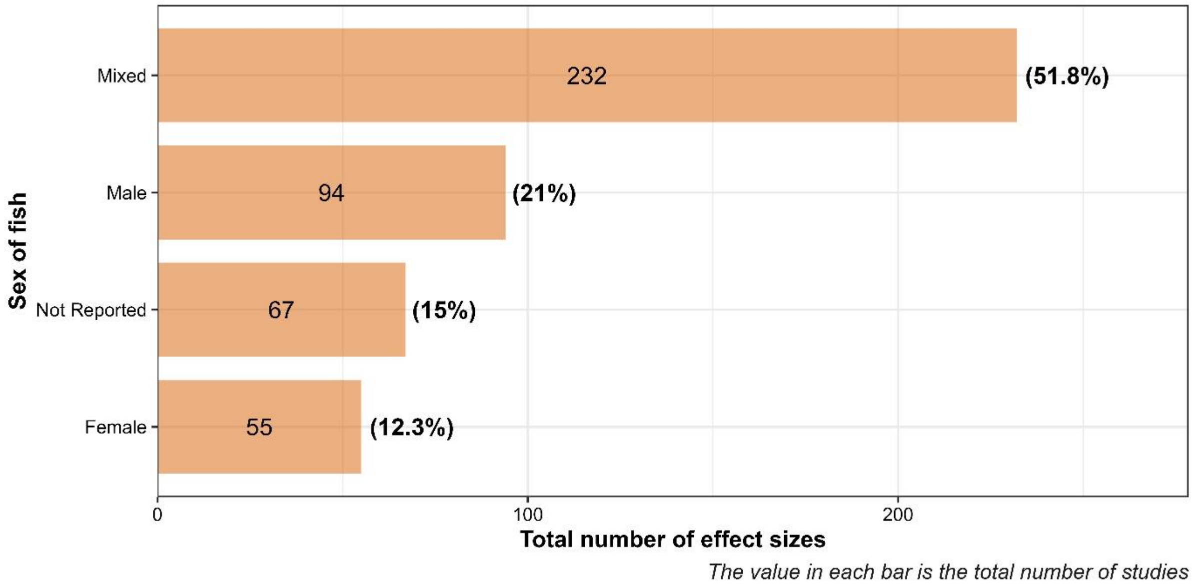

Figure s3) A bar chart showing the sex of fish used in the literature

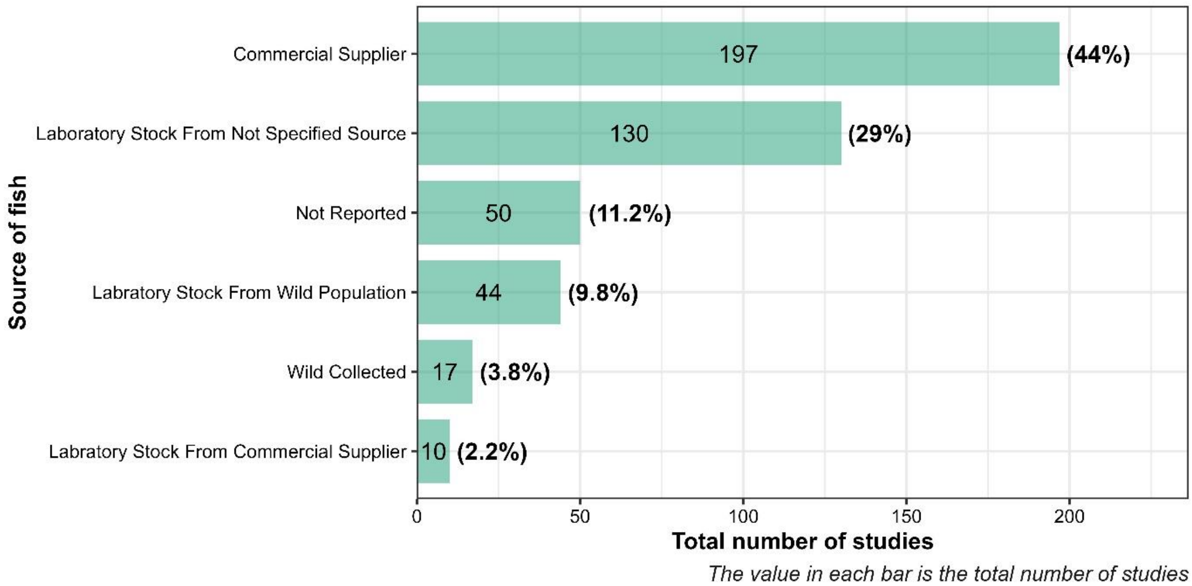

Figure s4) A bar chart showing the reporting of the source of fish used in the literature

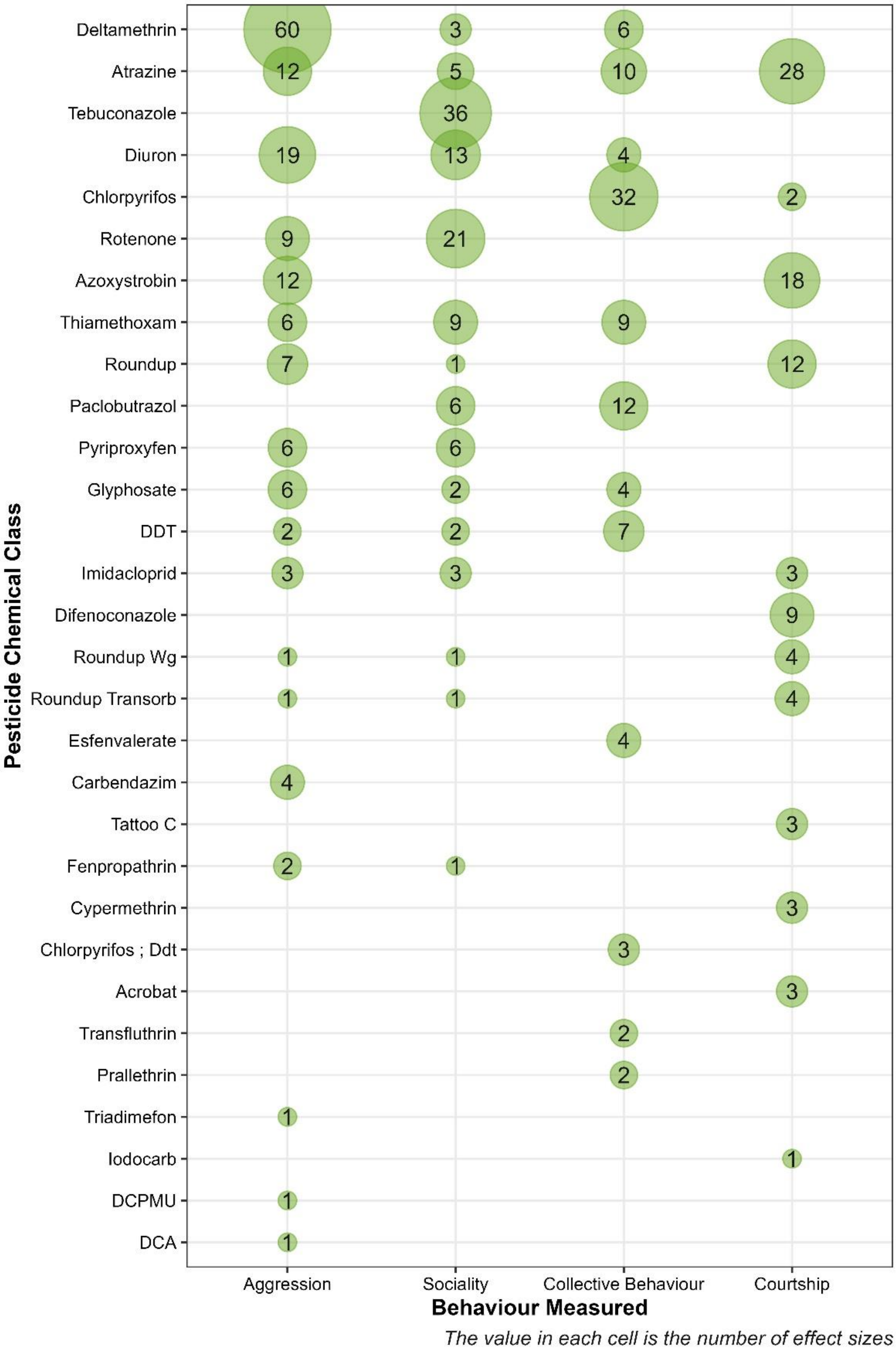

Figure s5) A bubble plot showing the number of effect sizes of each pesticide per conspecific interaction behaviour described

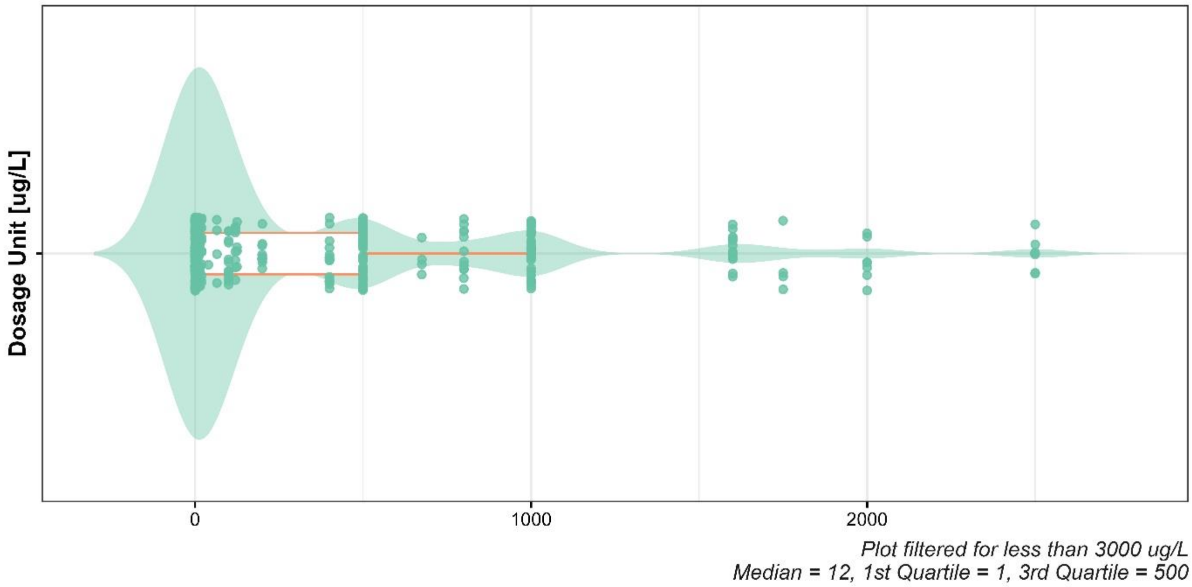

Figure s6) A box and violin plot showing the distribution of pesticide dosages used in the literature

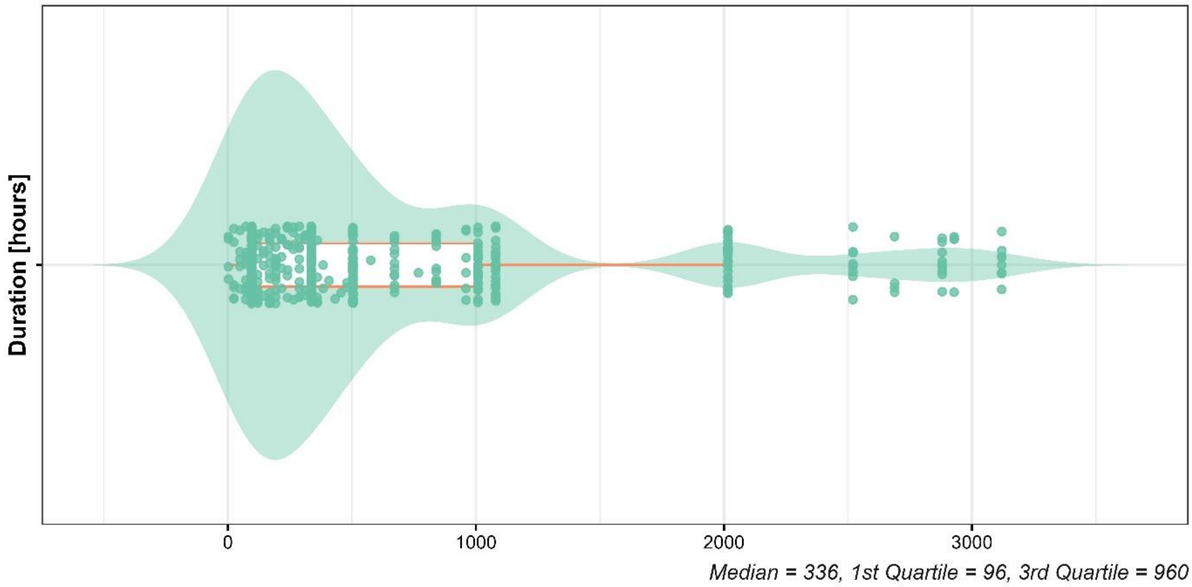

Figure s7) A box and violin plot the distribution of durations of pesticide exposure

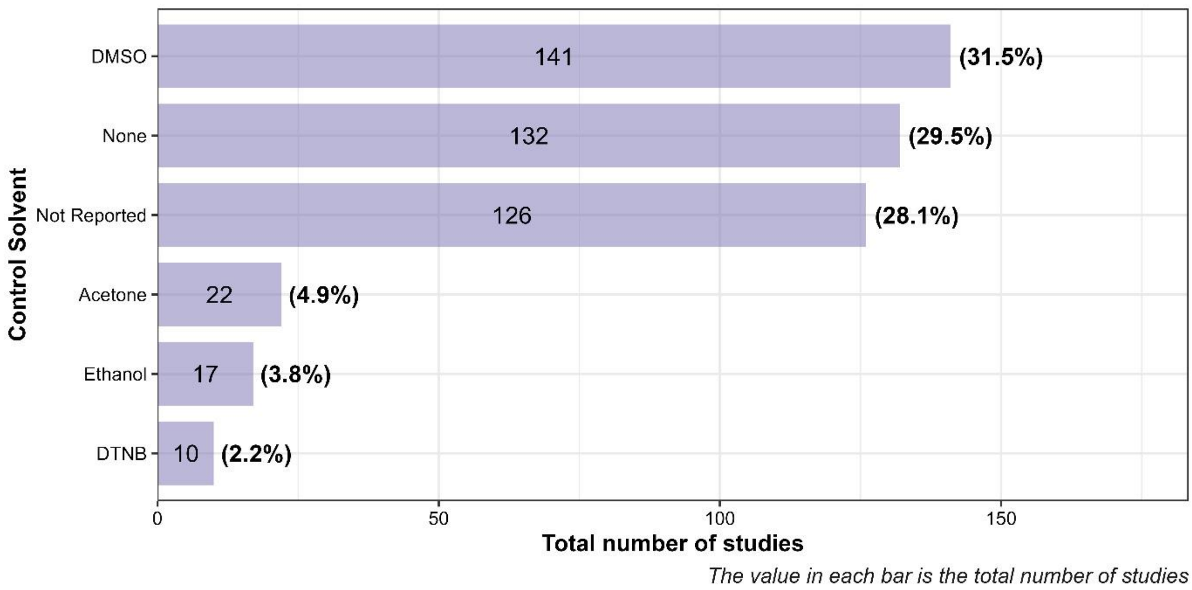

Figure s8) A bar plot showing the control solvents used in the literature

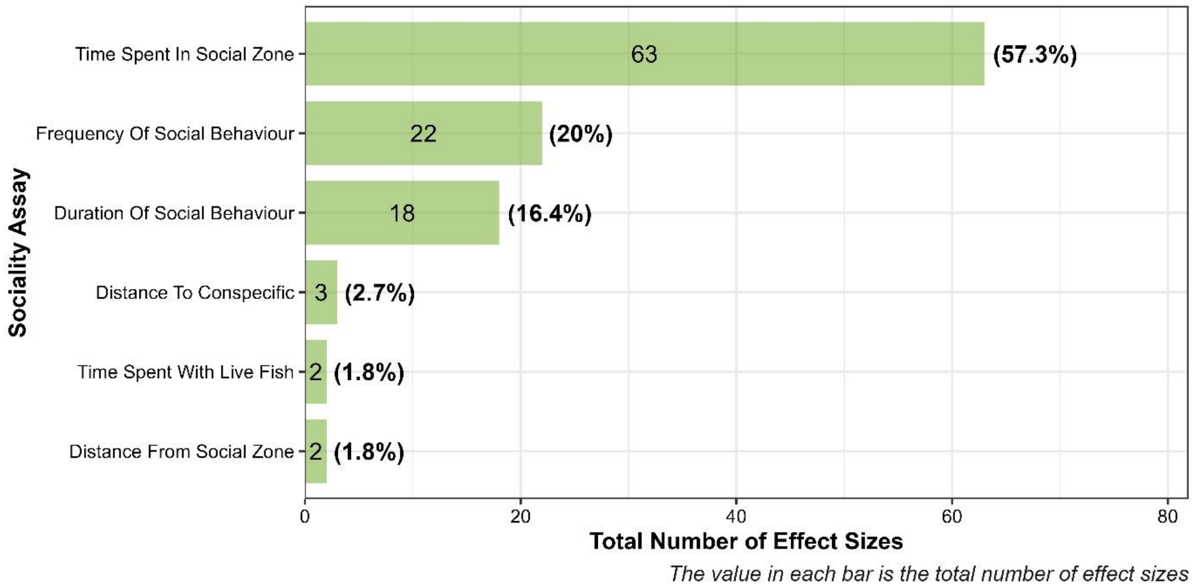

Figure s9) A bar plot showing the behavioural assays used to quantify sociality in the literature

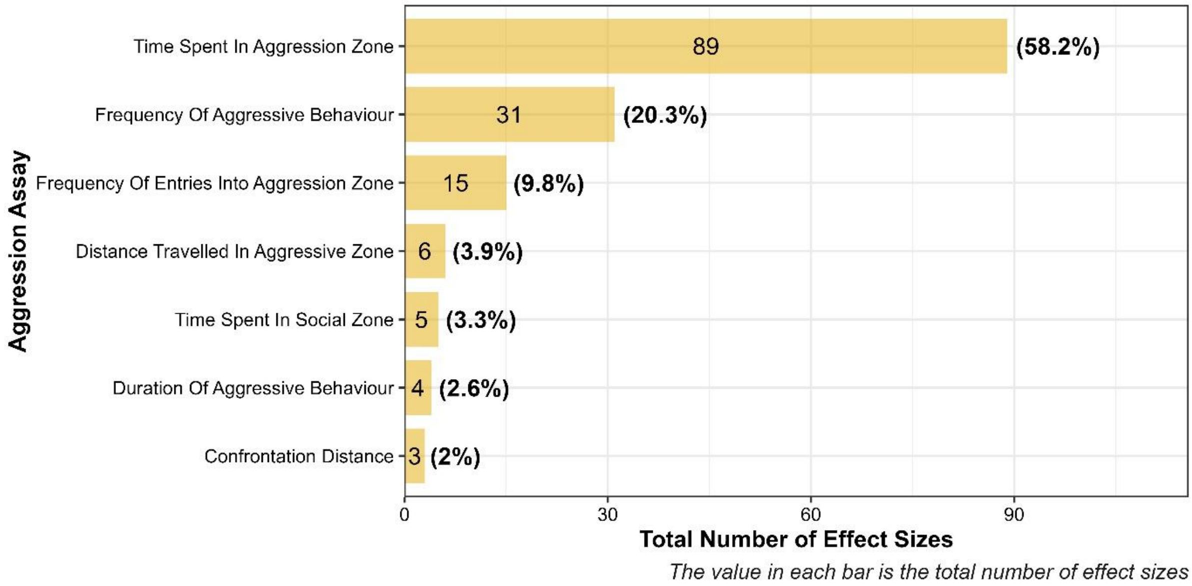

Figure s10) A bar plot showing the behavioural assays used to quantify aggression in the literature

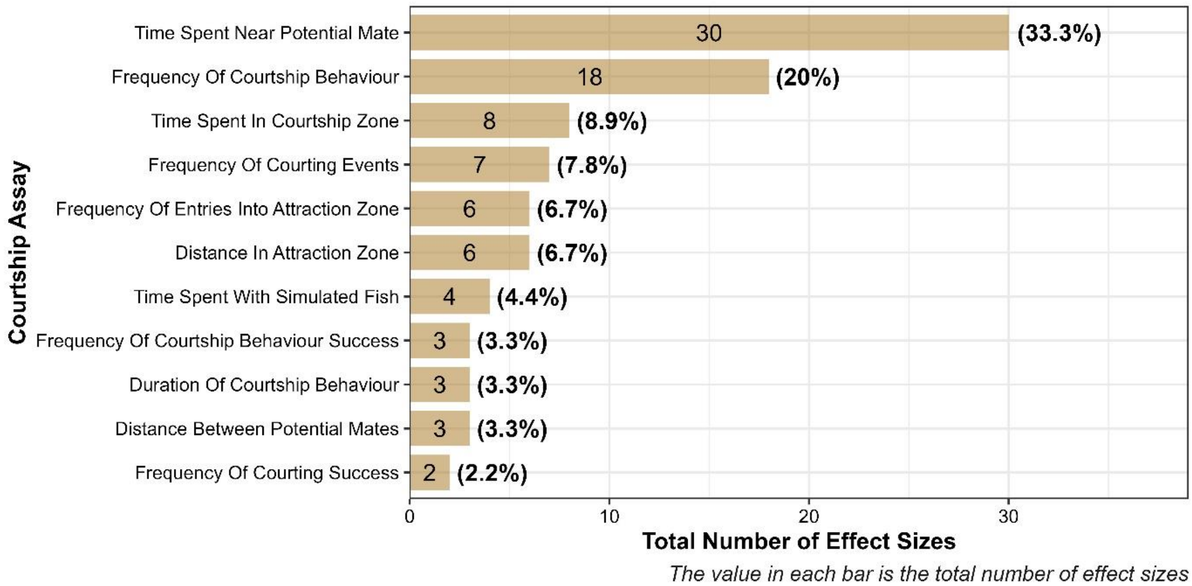

Figure s11) A bar plot showing the behavioural assays used to quantify courtship in the literature

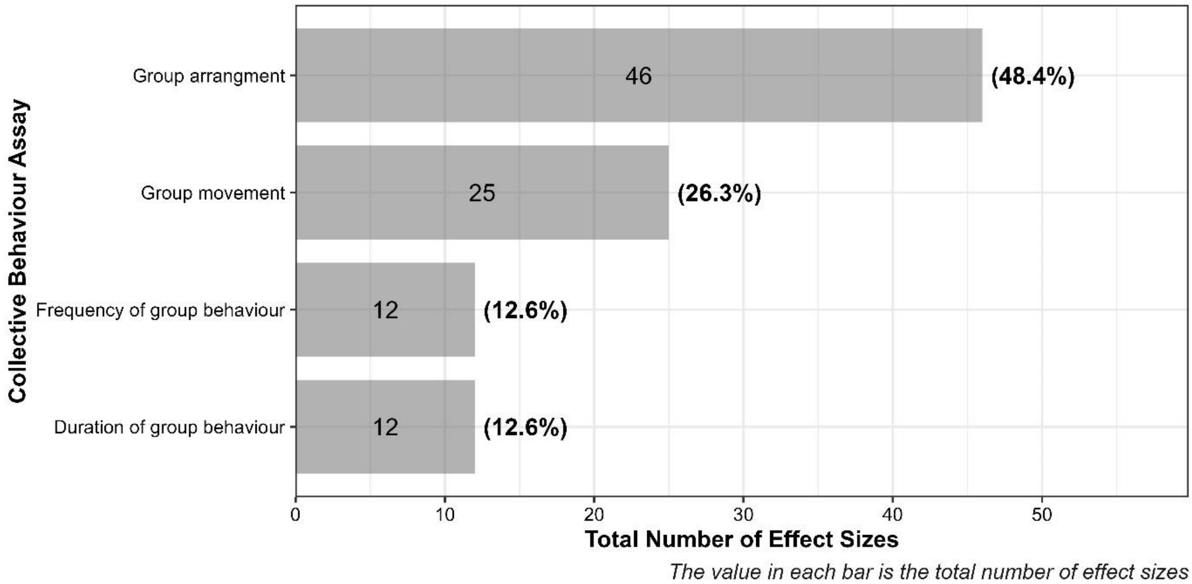

Figure s12) A bar plot showing the behavioural assays used to quantify collective behaviour (i.e., behaviours measure at collective level) in the literature

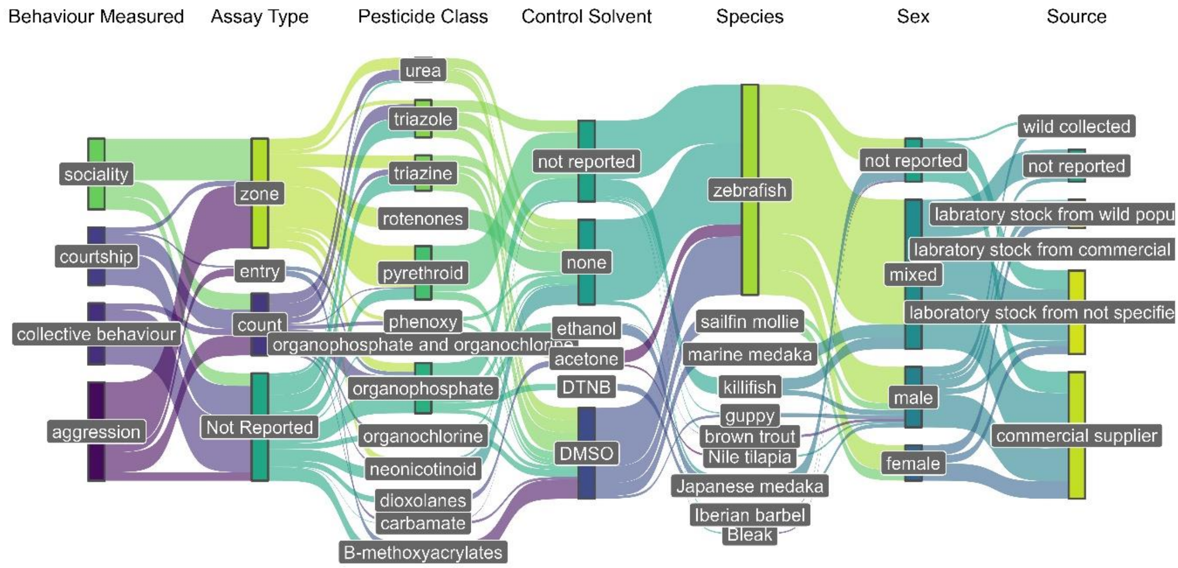

Figure s13) Visual representation of the characteristics of the primary studies included in the dataset. The vertical bars indicate key categorical variables. The widths of the vertical bars indicate the numbers of effect sizes represented by each level of the *categorical variable. The flow lines between vertical bars indicate the connections and overlaps of different levels of the categorical variables.*

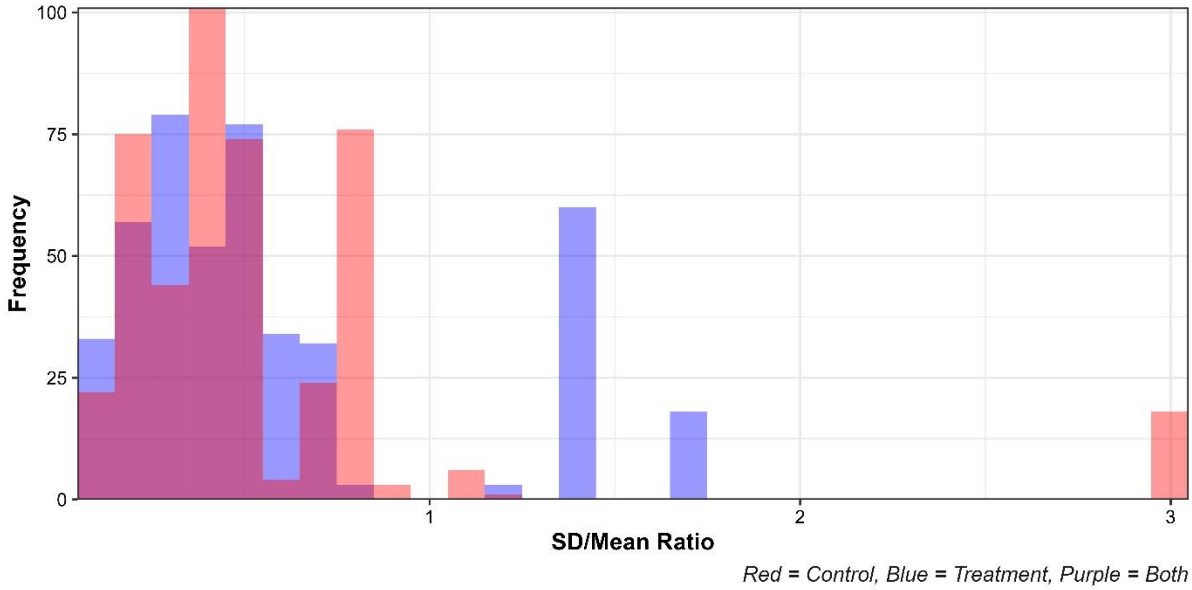

Figure s14) A bar plot showing the SD/Mean ratio of the control and treatment groups in each primary study

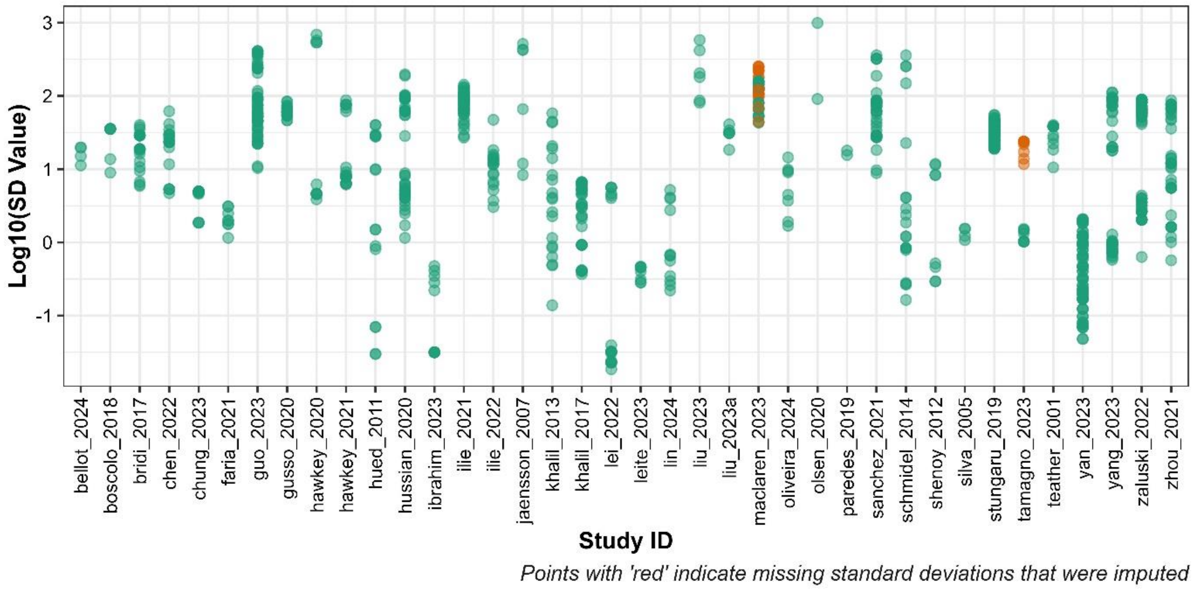

Figure s15) A plot presenting the impute SD values. We imputed the missing standard deviations using the formula provided in section 13.1 (p. 199) of the Handbook of Meta-analysis in Ecology and Evolution (Koricheva, Gurevitch, and Mengersen, 2013). To ensure consistency in treatments subjected to repeated stepwise multiple comparisons (i.e., the same data used multiple times to calculate different effect sizes), we maintained the same imputed standard deviations (or standard errors) for the repeated data.

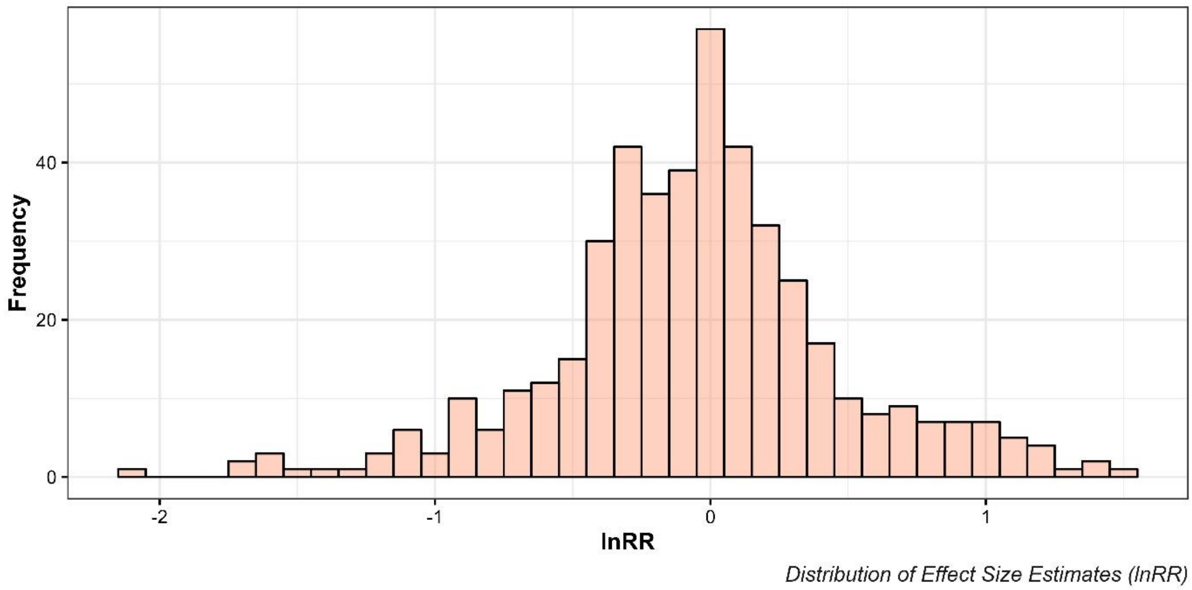

Figure s16) Histogram showing the distribution of lnRR estimates

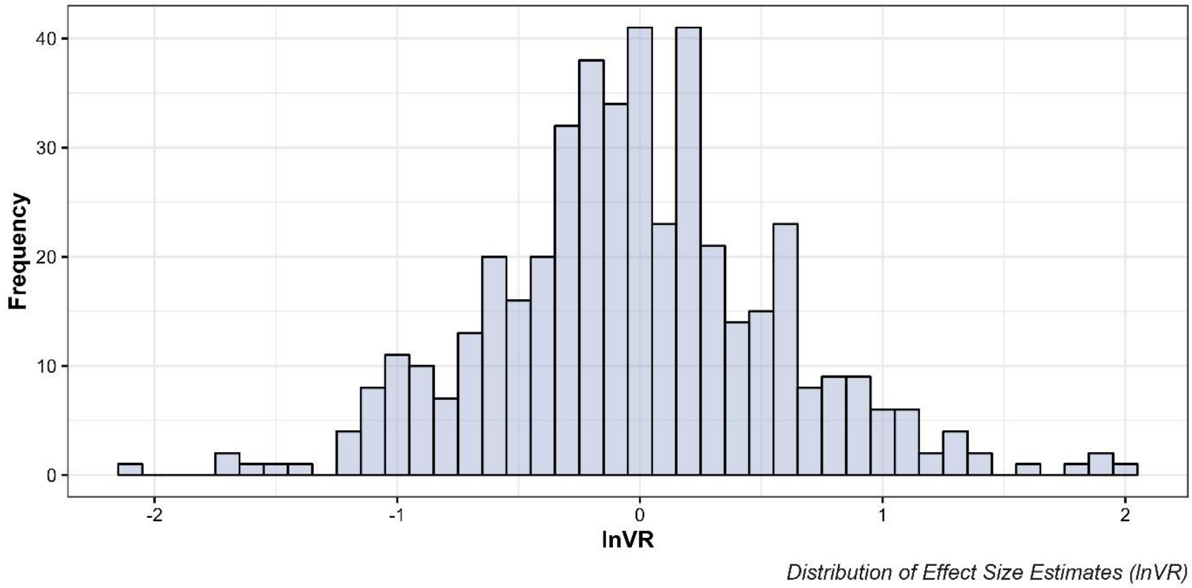

Figure s17) Histogram showing the distribution of lnVR estimates

Full pesticide and species characteristics orchaRd plots

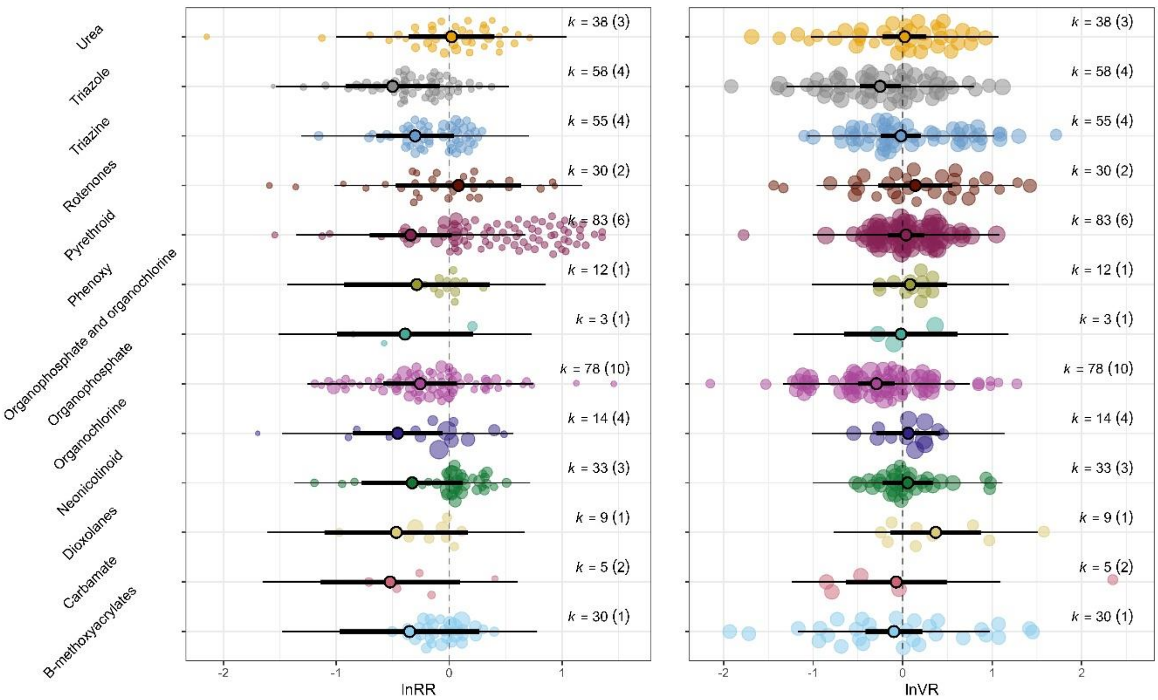

Figure s18) The moderating effects of pesticide chemical class on (A) response magnitude and (B) response variability in conspecific interactions. Shorter whiskers represent 95% confidence intervals, while longer whiskers indicate 95% prediction intervals. 'k' represents the number of effect sizes, and the number of studies is in brackets. Each circle corresponds to an effect size, with its size scaled according to precision (inverse sampling error variance).

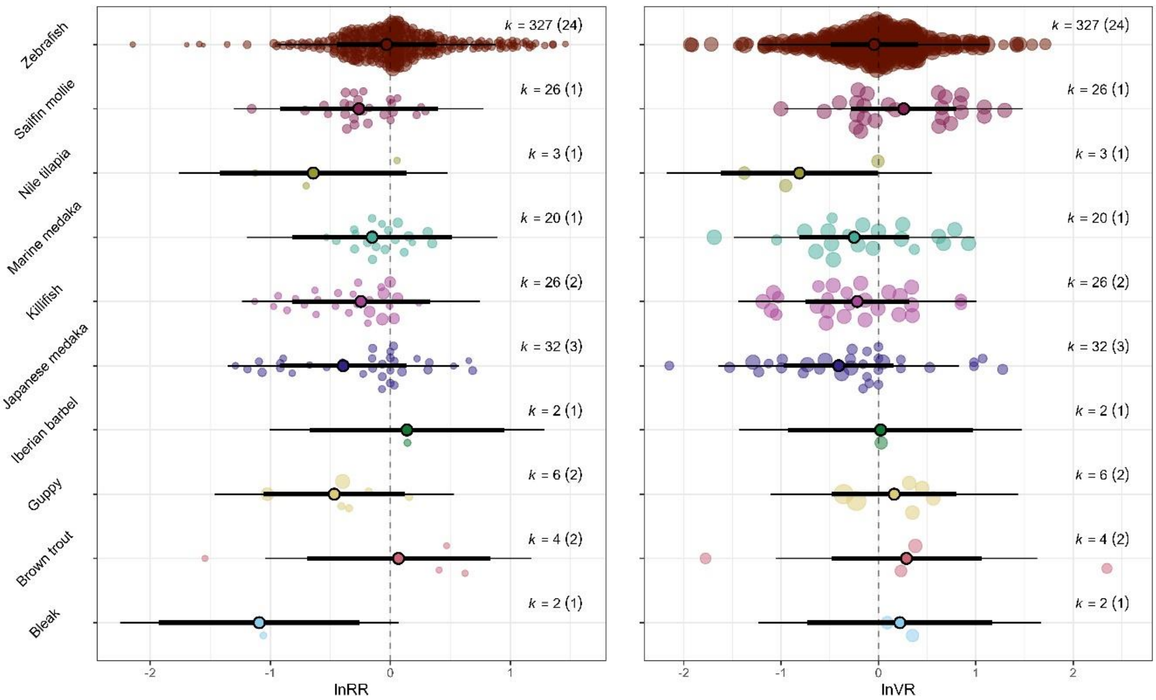

Figure s19) The moderating effects of species chemical class on (A) response magnitude and (B) response variability in conspecific interactions. Shorter whiskers represent 95% confidence intervals, while longer whiskers indicate 95% prediction intervals. 'k' represents the number of effect sizes, and the number of studies is in *brackets. Each circle corresponds to an effect size, with its size scaled according to precision (inverse sampling error variance).*

Publication bias, time-lag bias and sensitivity analysis

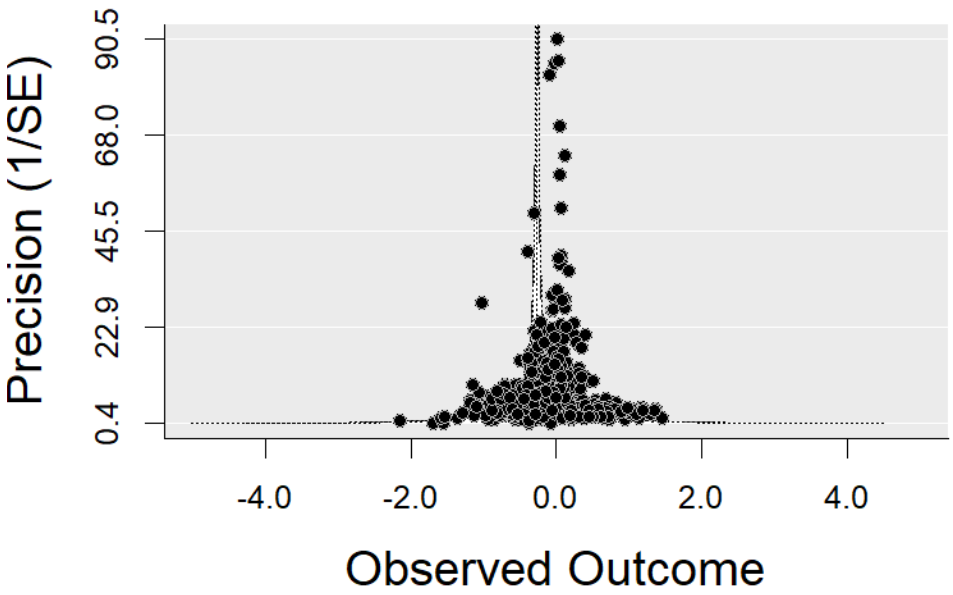

Figure s20) A funnel plot illustrating the distribution of study effect sizes in the meta-analysis. The symmetry of the plot suggests the absence of publication bias.

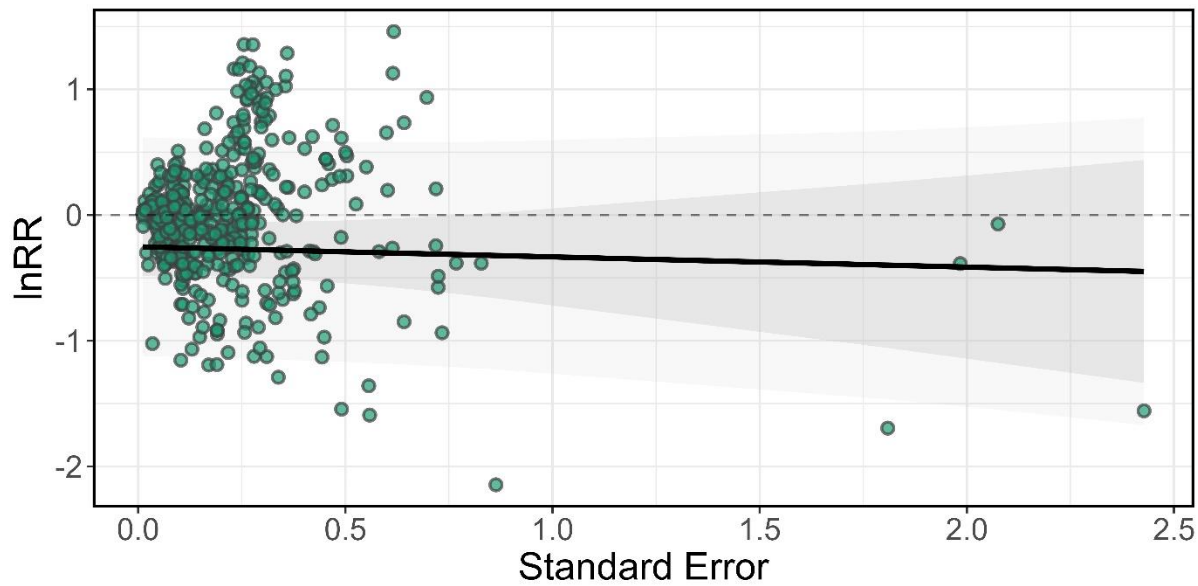

Figure s21) A plot showing the moderating effect of standard error on lnRR. The thick black lines show the prediction from the uni-moderator models with their associated 95% confidence interval (darker shaded area) and 95% prediction interval (lighter shaded area).

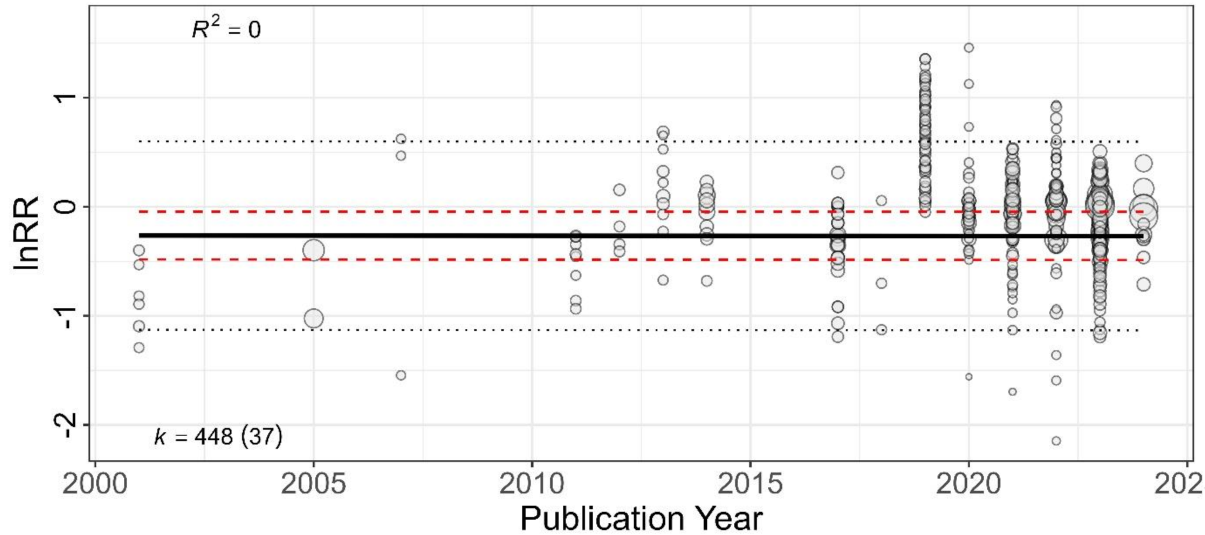

Figure s22) A bubble plot showing the moderating effect of publication year on lnRR. The thick black lines show the prediction from the uni-moderator models with their associated 95% confidence interval (red dotted line) and 95% prediction interval (black dotted line).

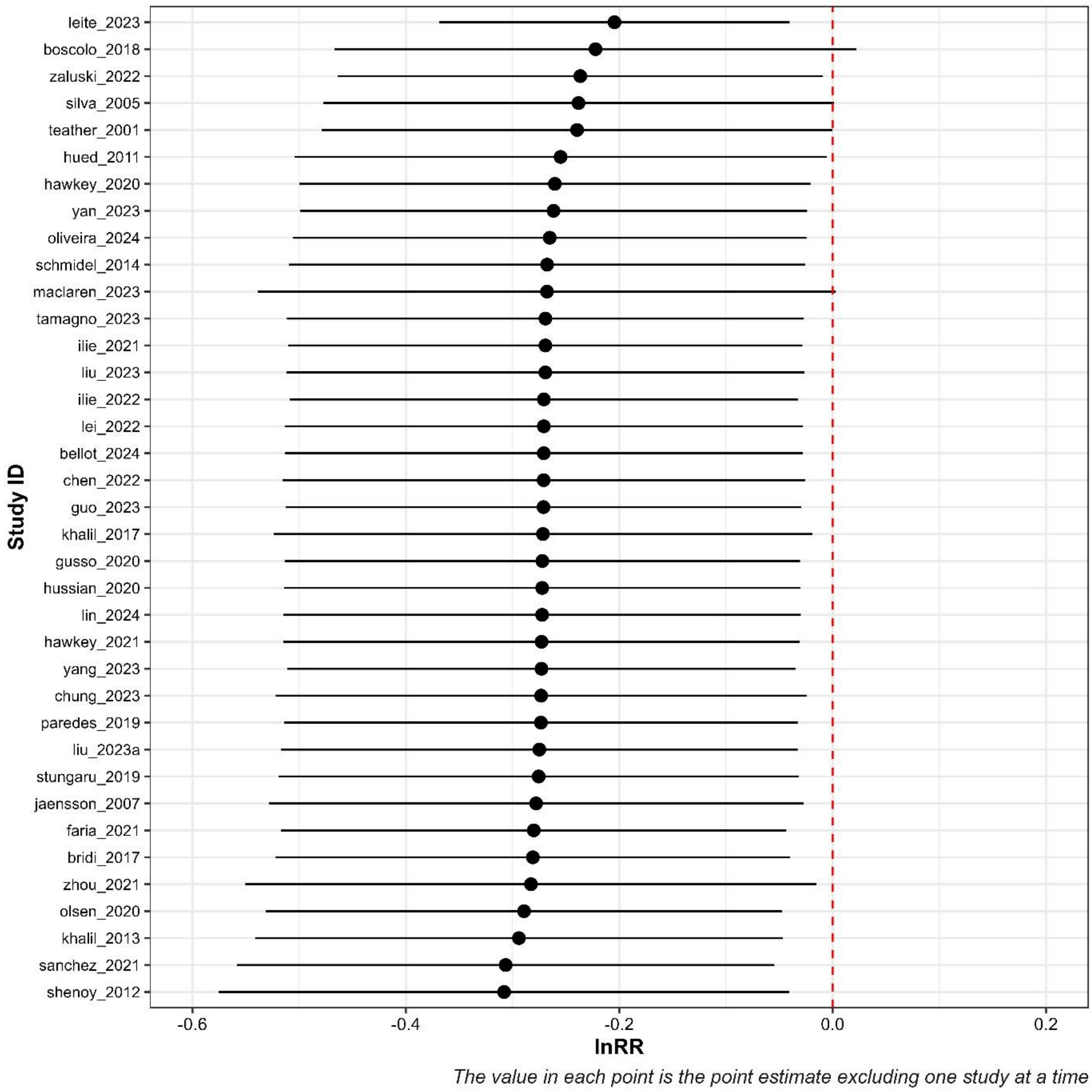

Figure s23) Forest plot displaying the results of the leave-one-study-out cross-validation of the meta-analysis model for lnRR effect size estimate. The solid dot represents the overall meta-analytic effect size estimate. Vertical lines represent 95% Confidence Interval.

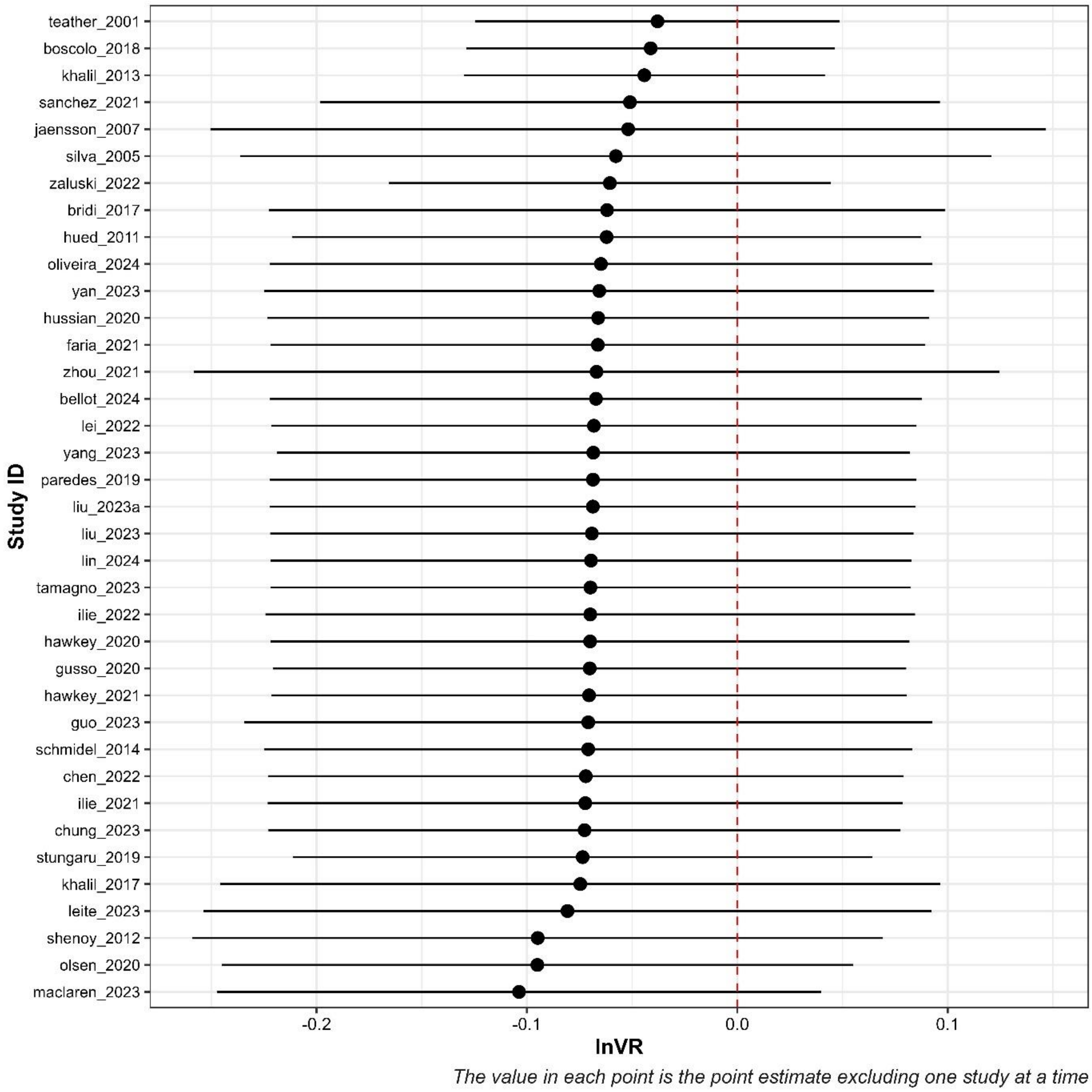

Figure s24) Forest plot displaying the results of the leave-one-study-out cross-validation of the meta-analysis model for lnVR effect size estimate. The rest of details are the same as Figure s21.

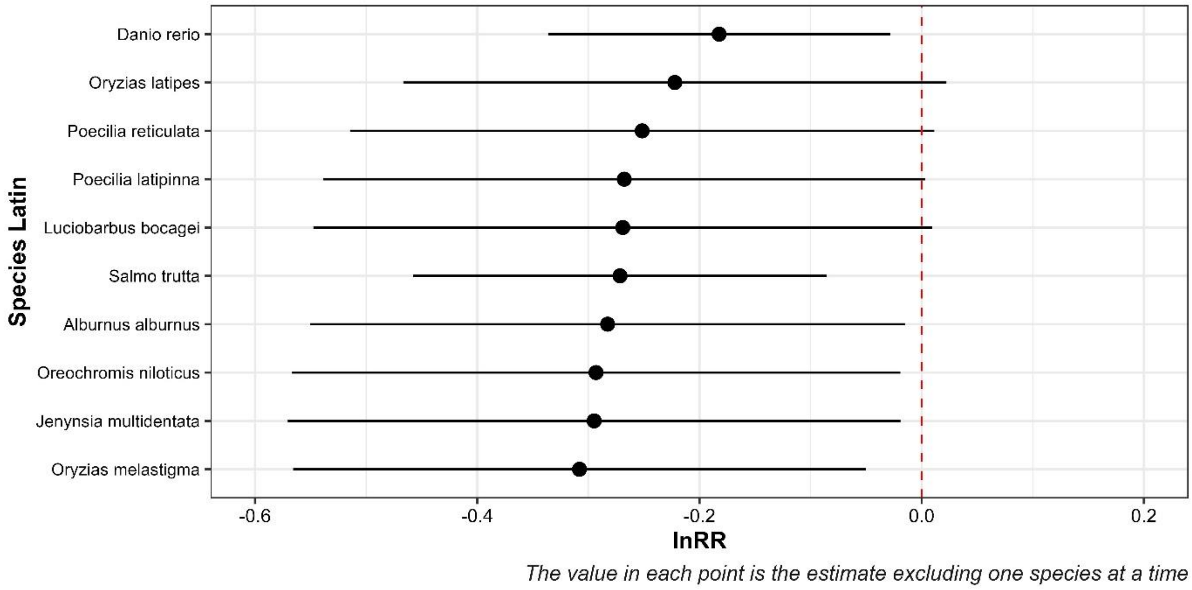

Figure s25) Forest plot displaying the results of the leave-one-species-out cross-validation of the meta-analysis model for lnRR effect size estimate. The rest of details are the same as Figure s21.

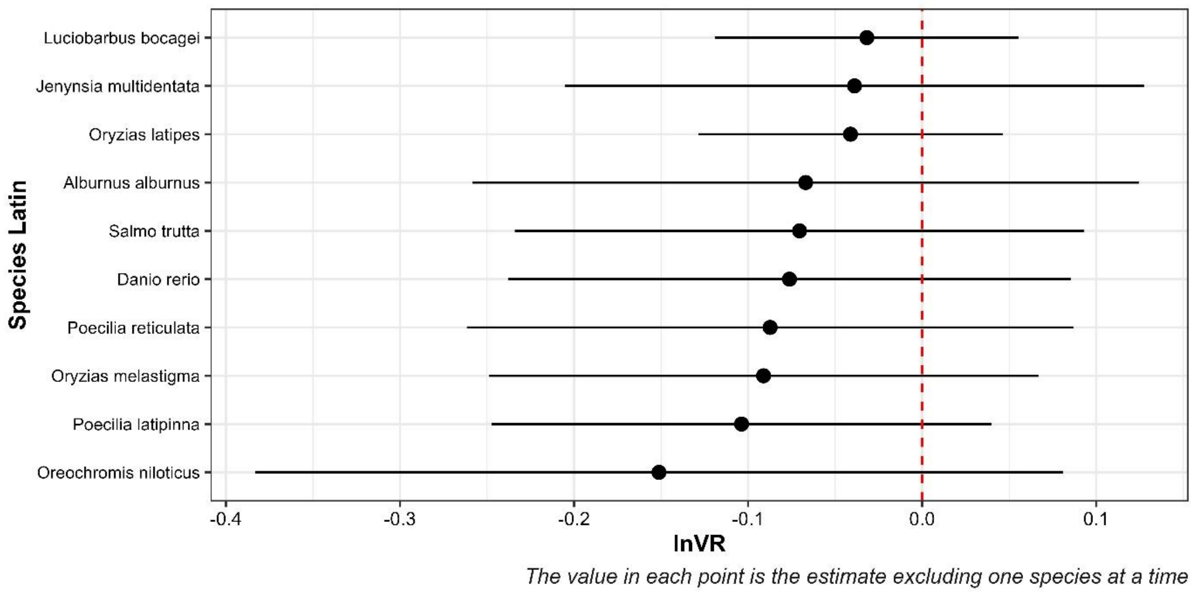

Figure s26) Forest plot displaying the results of the leave-one-species-out cross-validation of the meta-analysis model for lnVR effect size estimate. The rest of details are the same as Figure s21.

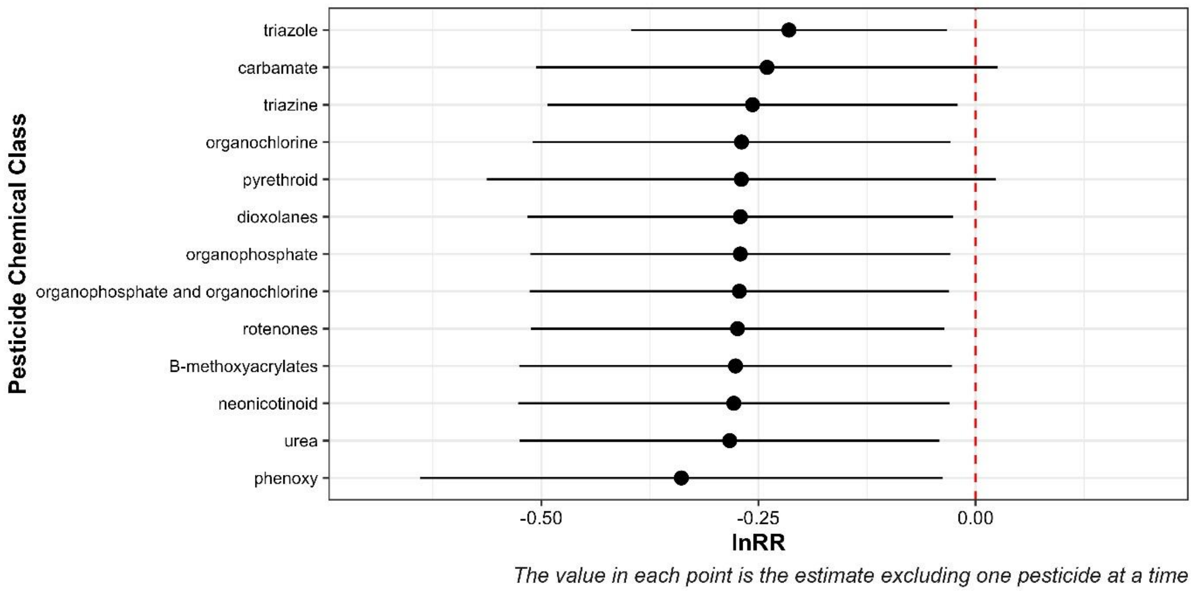

Figure s27) Forest plot displaying the results of the leave-one-pesticide-out cross-validation of the meta-analysis model for lnRR effect size estimate. The rest of details are the same as Figure s21.

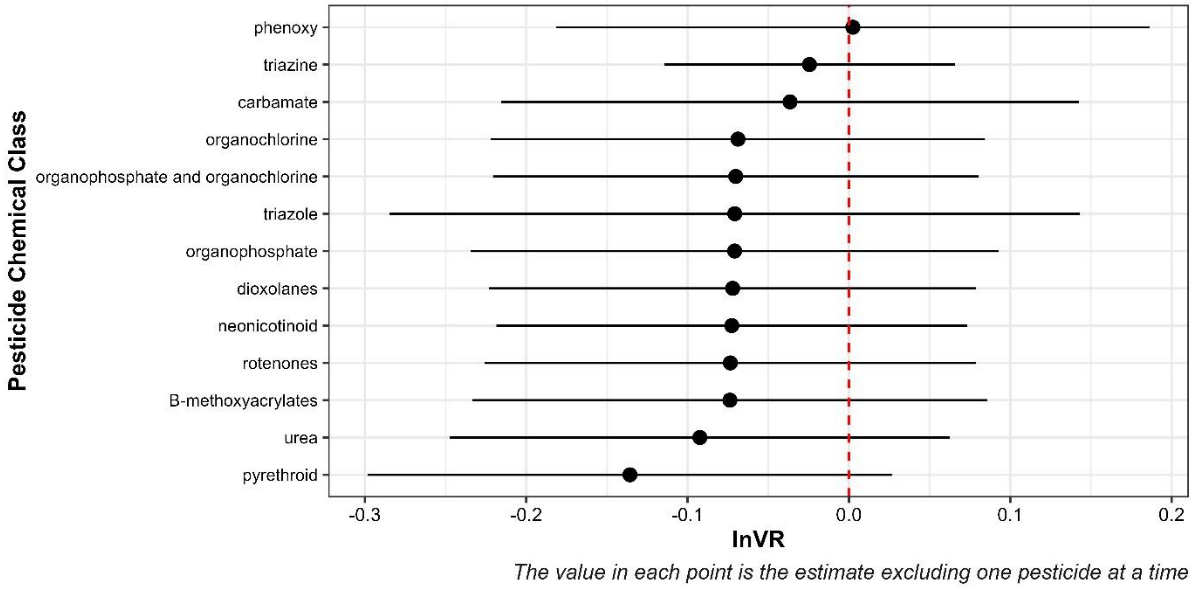

Figure s28) Forest plot displaying the results of the leave-one-pesticide-out cross-validation of the meta-analysis model for lnVR effect size estimate. The rest of details are the same as Figure s21.

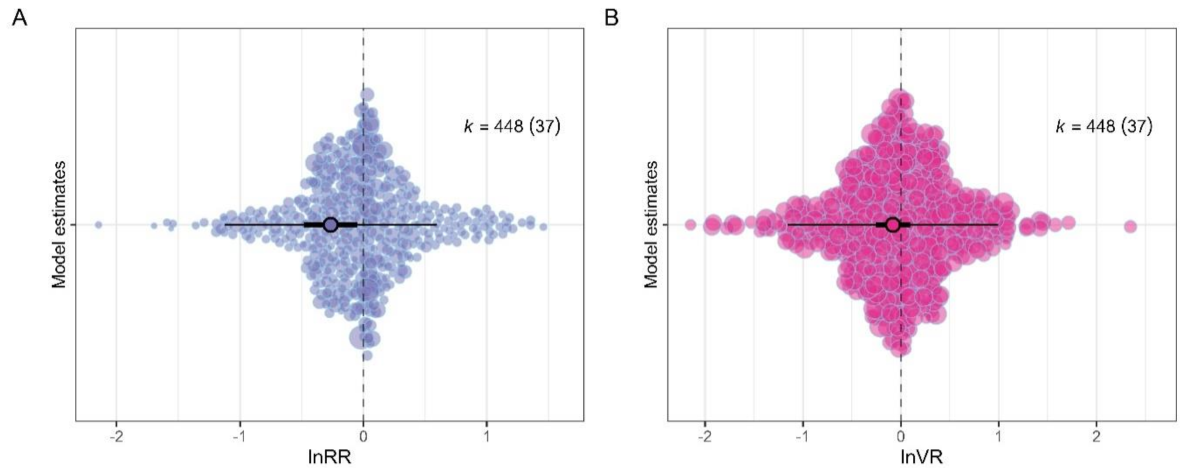

Figure s29) Impacts of pesticide exposure on fish conspecific interactions using alternative more conservative variance covariance matrix. (A) shows the mean difference between control and treatment groups on a logarithmic scale, where negative values indicate a reduction in conspecific behavioural activity. (B) shows the difference in variances between control and treatment groups, also on a logarithmic scale, where negative values suggest a reduction in the inter-individual variability of conspecific behavioural activity. Shorter whiskers represent 95% confidence intervals, while longer whiskers indicate 95% prediction intervals. 'k' represents the number of effect sizes, and the number of studies is in brackets. Each circle corresponds to an effect size, with its size scaled according to precision (inverse sampling error variance).*

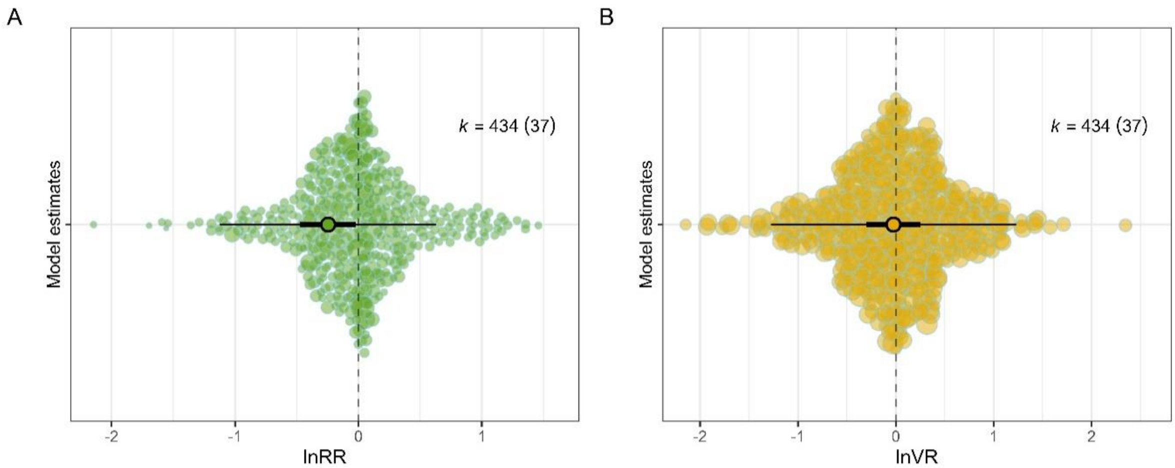

Figure s30) Impacts of pesticide exposure on fish conspecific interactions without imputed error estimates. The remaining details are the same as Figure s27.