**Protocol for Keum GLiTRS meta-analysis**

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**Table of content**

1.Abstract

2.Background

3.Objectives

4.Explanation of terms

4.1 Definition and background of threat

4.2 Definition and background of target taxa

4.3 Definition and background of biodiversity metric

4.4 Definition and background of geography

5. Methods

5.1 Search strategy

5.1.1 Data source

5.1.2 Search string

5.2 Deduplication

5.3 Screening criterion

5.3.1 Criterion of inclusion

5.3.2 Criterion of exclusion

5.4 Study quality assessment

5.5 Data extraction

5.6 Meta-analysis

6. Assessing bias

6.1 Publication bias

6.2 Reviewer bias

7. Sensitivity analysis

8. Reference

**1. Abstract**

This document provides a protocol for meta-analysis performed by Junghyuk Keum which examined the effect of insecticide application on Odonata abundance.

**2. Background**

The insects are globally declining. Anthropocene pressure is considered as the major cause. More and more empirical evidence is being found. Hallmann et al. (2017) claimed that flying insect biomass have declined approximately 76% in multiple areas of Germany during 1989~2016. Sánchez-Bayo and Wyckhuys (2019) claimed that within several decades, insects will decline by 40% across the globe. Such circumstances have grown concerns not only among academics but also the general public as well. The scientific community and the mass media are both warning about the catastrophic results of insect decline that will not only damage the ecosystem but the human society as well. For instance, in 2012, estimated 34.0 billion USD worth service was provided by the pollinator insects in USA (Jordan et al., 2021); various research are suggesting signs of decline in pollinator insects (Biesmeijer et al., 2006; Millard et al., 2021; Powney et al., 2021). However, unfortunately, the exact rate and status of the decline remains largely unknown. The situation is different among taxa, cause of decline, and region. Reports are difficult to integrate as they are fractured into limited regional data collections, each possessing different target taxa, methodology and arguments.

The GLiTRS is an international project run by the cooperation of multiple institutes across UK and South Africa. The aim of the project is to conduct a global threat-response model describing how the insect biome is responding to anthropogenic threats. By identifying individual response of particular insect taxa to specific threats in certain regions, we anticipate gaining global perspective of insect decline and figure out to which degree such phenomenon is predictable. In order to do that, the project plans to use methods such as meta-analysis, correlative relationships and collating expert opinions.

**3. Objectives**

I aim to aid the GLiTRS project by running a meta-analysis that addresses the effect of pesticide (i.e., insecticide) on global abundance of Odonatans. Therefore, the core question of this meta-analysis will be:

‘What is the effect of insecticide application on odonatan abundance globally’.

Threat: Insecticide

Target taxa: Odonata

Biodiversity metric: Abundance

Geography: Global

**4. Explanation of terms**

**4.1 Definition and background of threat**

Multiple evidence suggest that non-target arthropods and insects are being harmed by the use of insecticides. For instance, butterfly abundance and species richness were found to be negatively correlated with the use of pesticides on farmlands in Europe and North America (Forister et al., 2016; Habel et al., 2019). Main et al., (2018) demonstrated in their meta-analysis that various arthropods are being harmed by the use of insecticide.

Here, I defined an insecticide as a pesticide that is designed to specifically target insects. Other forms of pesticide such as herbicide, nematicide, molluscicide and rodenticide were excluded.

**4.2 Definition and background of target taxa**

The order Odonata is one of the insect groups that requires a global biodiversity assessment. Composed of 2 subgroups, dragonflies and damselflies, odonatans are carnivorous insects found on every continent excluding Antarctica (Hassall & Thompson, 2008). They possess a unique life cycle, with both aquatic and terrestrial life-history stages, making them ideal ecological indicators (Kalkman et al., 2010). As larvae (nymph), they prefer shallow freshwater bodies such as rice paddies, swamps, and ponds. After molting into adult (imago), they spend most of their time flying through vegetation preying on smaller insects. As obligate predators, odonatans also provide us with pest controlling services, feeding on organisms that hinder rice growth and mosquitoes (Fincke et al., 1997; Ghahari et al., 2009; Mandal et al., 2008; Jacob et al., 2017). However, various evidence suggest that odonatans are being harmed by the use of pesticides (Martins, 2009; Tazunoki et al., 2022; Chang et al., 2007). Furthermore, the ecology of odonatans is still largely unknown. For instance, even though odonatans should be considered as major conservation priorities as 106 out of 118 species of endangered aquatic insect species listed on the IUCN red list are oodonatans (Kalkman et al., 2010), 35% of all odonatan species registered in the IUCN red list are still classified as ‘data deficient’ in terms of how threatened they are, which is higher than the average of 27% for all (9,355 species) threatened insect species on the IUCN red list (Clausnitzer et al., 2009; Wang et al., 2021). This might be due to the fact that studies are mostly based on regional observations working on certain species and conditions, lacking an integrated general result. For example, even though 60% of Odonata species richness is found in neotropical and indo-malayan regions (Clausnitzer et al., 2009), Grames et al. (2022) demonstrated that 68% of recent studies examining Odonata biodiversity were conducted in either Europe or North America while none were from South America and Oceania. Fortunately, freshwater invertebrates are highly responsive to conservation managements (Outhwaite et al., 2020). Therefore, understanding how odonatans are reacting to pesticides will aid us in planning future conservation strategies.

**4.3 Definition and background of biodiversity metric**

I defined abundance as the number of Odonatans individuals present in a given study area, sampling device, etc.

**4.4 Definition and background of geography**

I collated data from around the globe. I recorded country, longitude and latitude of the study when possible.

**5. Methods**

My meta-analysis will be conducted in the following steps. First, I will search for studies that compare Odonatan abundance between areas with and without insecticide application. Then, a screening process will be done in order to leave only the studies of interest. Data will be extracted from those studies and will be recorded in a separate Excel datasheet. The data will be then used to run a meta-analysis using the metafor R package (Viechtbauer, 2010) in R software. Followed by a sensitivity analysis and a bias assessment to ensure the quality of the meta-analysis.

**5.1 Search strategy**

**5.1.1 Data source**

I will conduct our search of literature from the following database:

Scopus

Web of Science

**5.1.2 Search string**

I will conduct our proto search string according to the PICO (Population, intervention, Comparison, Outcome) elements.

Our PICO elements are as following:

P: Odonata

I: Insecticide

C: Comparison

O: Abundance

Therefore, our proto search string will be as following:

Proto search string:

(e.g., (Odonata\* OR Dragonfl\* OR Damselfl\*) AND (Pesticide OR insecticide) AND (Compar\* OR Contrast\*) AND (Abundance OR richness OR Occupancy)

In order to record the process of conducting our search string, I will conduct a flow chart of how many hits each search string returns and how many relevant papers are found in the first 20 papers.

After iterative process of adding and excluding terms to maximize the number of relevant papers found, I will finalize our final search string which returns the highest numbers of relevant papers in the first 20 papers.

**5.2 Deduplication**

I will perform deduplication process according to Bramer et al., (2016), which involves using the ‘Find Duplicates’ function in software Endnote.

**5.3 Screening criterion**

In order to acquire only the study of interest, I will perform 3 rounds of screening process. 1st round, I will skim through titles and abstracts of papers to exclude obviously irrelevant papers (e.g., paper only briefly mentioning Odonata). 2nd round, I will run through titles and abstracts again but with more precision according to the inclusion criteria to leave studies of interest. 3rd round, I will perform a full text screening to exclude studies that failed to satisfy the criteria.

After screening, I will record the whole process of how many papers were found and how many were left after every round using a PRISMA diagram (Page et al., 2021). Reason of exclusion will be recorded for excluded studies.

**5.3.1 Criteria of inclusion**

|  |
| --- |
| 1. Paper must compare Odonata abundance between areas with and without insecticide application. 2. Paper must be written in English. 3. Paper must be primary research. Any form of non-primary research will be excluded (e.g., review, article, meta-analysis). 4. Paper must address at least one species of Odonata as their target taxa. 5. Paper must contain mean Odonata abundance, standard deviation, and sample size (n > 1) for each area with and without insecticide application. 6. Studied insecticide should be either referred to as a pesticide, insecticide, or larvicide. The type and concentration of insecticide will not be considered. Papers examining the effect of other forms of pesticide (e.g., herbicide, nematicide, molluscicide, rodenticide) will be excluded. |
| 1. Study area should be either agricultural area or pristine (wild) area. 2. Papers must involve field experiments (including mesocosms) observing naturally occurring odonatans. |

**5.4 Study quality assessment**

I assessed the quality of the studies following these standards:

Here, I define high-quality comparisons as the following: comparisons that (1) involve sample size larger than 5 (≥ 5), (2) involve insecticide application area and control area that are within close (≤ 1km) distance (so that they have similar environments), (3) involve simultaneous observation/sampling events in both area, (4) observe nymphs or exuviae as indicators of Odonata abundance rather than adults (i.e., adult odonatans might have flown in from a discrete area). Comparison that fails to satisfy all 4 criteria will be considered low-quality. I will then run the analysis without the low-quality comparisons to verify whether my results are overly affected by study quality. When information is insufficient to make judgements (e.g., distance between areas not provided), I will only assess given information. For low-quality comparisons, I will record the reason for the assignment of that quality.

**5.5 Data extraction**

I will extract bibliographic information, qualitative information and quantitative information from final papers according to the data input spreadsheet (for details see datasheet in appendix).

Bibliographic information:

Literature ID, Study ID (in case multiple studies or comparisons exist within one literature), Title, Author, Year, Journal, Volume, Pages, URL, DOI, Database

Qualitative information:

Order, Family, Species, Threat type, Threat name, Country, Latitude, Longitude, Experimental year, Agriculture, Life stage, Sampling method, Biodiversity metric, Unit, Extraction source, Additional biodiversity metric, Study quality.

Quantitative information:

Mean Odonata abundance, standard deviation, and sample size for each pesticide application and control area.

When required data is only given within figures, I will use metaDigitise (Pick et al., 2018) R package to extract data.

**5.6 Meta-analysis**

I will perform my meta-analysis using the metafor (Viechtbauer, 2010)) R package.

I will first calculate the effect size and measurement of variance for each comparison from the quantitative information that I extracted, using the escalc function in the metafor R package (Viechtbauer, 2010). The effect sizes will be generated as log response ratios. As the log response ratio cannot be calculated when either the mean Odonata abundance of the pesticide application area or the control area are 0, I will add 10 values from 0.1 to 1 with an interval of 0.1 (i.e., 0.1, 0.2, 0.3, … 1) to every mean value in my data set and examine how added values affect my overall results.

Using the effect sizes and measurement of variance, I will then perform a random effect model meta-analysis to examine the effect of insecticide on Odonata abundance. As multiple effect sizes will be drawn out from the same source, I will use the rma.mv function of the metafor package to account for the non-independence within papers.

I will conduct all statistical analysis using program R (v. 4.1.1, R Core Team, 2021).

**6. Assessing bias**

**6.1 Publication bias**

When there are more than 10 papers (Borenstien, 2011), I will conduct a funnel plot using the funnel() function from the metafor R package in order to detect possible publication bias within the papers.

I will then perform an Egger’s test using the regtest function in the same R package, to assess whether my funnel plot is indicating a significant degree of publication bias (Egger et al., 1997; Viechtbauer, 2010).

**6.2 Reviewer bias**

I will perform a Kappa test (Galton, 1892) in order to assess reviewer bias (in case more than one reviewer).

**7. Sensitivity analysis**

I will perform a sensitivity analysis on the results by running the meta-analysis again excluding low quality papers.

**8. Reference**

Biesmeijer, J.C., Roberts, S.P., Reemer, M., Ohlemuller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J. & Kunin, W.E. (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science, 313, 351-354.

Borenstein, M.H.L.V.H.J.P.T.R.H. (2011) Introduction to meta-analysis.

Bramer, W. M., Giustini, D., De Jong, G. B., Holland, L., & Bekhuis, T. (2016). De-duplication of database search results for systematic reviews in endnote. Journal of the Medical Library Association, 104(3), 240–243. <https://doi.org/10.3163/1536-5050.104.3.014>.

Galton, F. (1892) Finger Prints, Macmillan, London.

Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Muller, A., Sumser, H., Horren, T., Goulson, D. & de Kroon, H. (2017) More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS One, 12, e0185809.

Jordan, A., Patch, H.M., Grozinger, C.M. & Khanna, V. (2021) Economic Dependence and Vulnerability of United States Agricultural Sector on Insect-Mediated Pollination Service. Environ Sci Technol, 55, 2243-2253.

Millard, J., Outhwaite, C.L., Kinnersley, R., Freeman, R., Gregory, R.D., Adedoja, O., Gavini, S., Kioko, E., Kuhlmann, M., Ollerton, J., Ren, Z.X. & Newbold, T. (2021) Global effects of land-use intensity on local pollinator biodiversity. NAT COMMUN, 12, 2902.

Pick, J.L., Nakagawa, S., Noble D.W.A. (2018) Reproducible, flexible and high-throughput data extraction from primary literature: The metaDigitise R package. Biorxiv.  
https://doi.org/10.1101/247775.

Powney, G.D., Carvell, C., Edwards, M., Morris, R.K.A., Roy, H.E., Woodcock, B.A. & Isaac, N.J.B. (2019) Widespread losses of pollinating insects in Britain. NAT COMMUN, 10, 1018.

Sánchez-Bayo, F., Wyckhuys, K. A. G. (2019) Worldwide decline of the entomofauna: A review of its drivers. Biological Conservation, 232, 8-27.

Viechtbauer, W. (2010). Conducting meta-analyses in R with the metafor package. Journal of Statistical Software, 36(3), 1-48. https://doi.org/10.18637/jss.v036.i03.