



Insecticides and dragonflies



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Tutorial for water sampling and selection of transects

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Abstract	This document describes how transects from the Dutch Dragonfly Monitoring Scheme are to be selected for sampling and how water samples should be collected and stored.
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1. Introduction

This document gives instructions on how to select suitable transects from the Dutch Dragonfly Monitoring Scheme to study the impact of pesticides on dragonflies and how samples should be taken and treated. This is a part of the ACTION-pilot “insecticides and dragonflies”. The goal of this pilot is to gain insight into the impact of pesticides on dragonfly communities.

Dutch Butterfly Conservation (<https://www.vlinderstichting.nl/english>) is a non-profit organisation that studies butterflies, dragonflies and moths in order to advise management organisations and better protect these species. One of the core activities is the monitoring of these groups, this is primarily done with citizen scientists that count animals according to a standardized protocol. To understand why certain species or groups of species are doing poorly additional research is done. This research can be independent of the monitoring, even if it is inspired by the monitoring results, or incorporated in the monitoring. In the latter case we can use the wealth of data the monitoring program provides. In the current proposal we will do that latter and measure pesticides on locations where dragonflies are being monitored

(<https://www.vlinderstichting.nl/wat-wij-doen/meetnetten/meetnet-libellen/>). The feedback we had so far does indicate that many citizen scientists would like to contribute. We will ask for people to volunteer to do this through our newsletter, at the annual meeting and by directly asking people that count suitable transects.

This is a citizen science pilot study of the H2020-SwafS-2018-1-824603 EU project. More info about the project at <https://actionproject.eu/>

2. Insecticides and dragonflies

Insecticides and other pesticides are widespread in the environment. These compounds are all tested in laboratories but their impact under natural conditions are poorly known. In typical conditions multiple compounds can be found at the same time and may interact and the environment is far from those in laboratory studies. In the recent study by Barmantlo et al. (2019) we found that, under natural conditions, damselfly larvae were negatively affected by low concentrations of the neonicotinoid thiacloprid.

Since 1999 Dutch Butterfly Conservation has a monitoring program for dragonflies where dragonflies are counted along transects by citizen scientists following a protocol. This is done on more than 500 transects a year and allows us to calculate trends in the abundance of dragonflies in the Netherlands. We would like to build on this data to research whether there is a correlation between pesticide presence in water and dragonfly prevalence. Our transects are in nature reserves but also in parks and agricultural areas. This allows us to select locations that differ in their exposure to pesticides. Samples of water will be collected from these sites by the same citizen scientists who count the dragonflies. Testing of the samples for pesticides requires sophisticated laboratory equipment, and is carried out by students at the University of Applied Sciences in Leiden.

*Barmantlo, S. H., Vriend, L. M., van Grunsven, R. H. A., & Vijver, M. G. (2019). Environmental levels of neonicotinoids reduce prey consumption, mobility and emergence of the damselfly *Ischnura elegans*. Journal of Applied Ecology, 56(8), 2034-2044.*



Figure 1. Blue tailed damselfly is declining in the Netherlands and exposure to pesticides is a possible cause of this decline (Photo: Kim Huskens).

3. Selection of transects

There are some key aspects of transects that have to be considered when deciding which transects should be sampled. In this way it will be practical, the data can actually be used and we avoid biases. The selection of transects will be done with citizen

scientists that indicate that they want to contribute. There are some criteria that make transects more or less suitable.

- First of all we need to have transects where the citizen scientist is willing to take water samples for this project. The effort is relatively small compared to the transect counts and can be done simultaneously and it gives additional insight in the local conditions. We therefore think that this will not be a problem.
- The transects should have sufficient data to make comparisons possible, they should at least be counted regularly over the last three years. Transects that just started or have only been counted infrequently may give a less accurate estimate of the local dragonfly populations.
- Habitats that only occur in nature reserves are excluded. These are for instance peatbogs, heathland ponds, mesotrophic fenns etc. These types of habitats are rich in dragonflies and are unlikely to be exposed to high concentrations of pesticides. However, these two factors are not related. We only include habitats that do occur both inside and outside of nature reserves and thereby avoid this confounding effect.

4. Water sampling

The actual sampling by the Citizen Scientists is done when they are in the field to count dragonflies. Therefore there is no additional travel time involved. We will supply suitable containers, 200 ml tubes with screw caps, and ask the volunteers to sample the same location every time they count dragonflies. They should record the location, preferably take a photo, hold the container under water, a few centimeters below the surface and at least 10 centimeters away from the waters edge, in this way the sampled water will be representative. On each container the following things should be noted with waterproof marker after the sample is closed:

- Date
- Transect name
- Transect number
- Section number
- Name of the person taking the sample

A photo of the sample location should be provided when possible. The container should be stored out of the sun and frozen as soon as possible. The samples will be collected for analysis and brought to the lab frozen. In the lab all samples will be numbered and the other information stored in a spreadsheet with the number of the sample.

Communication with the citizen scientists is well organised as they already participate in the monitoring scheme. We therefore regularly send them newsletters and many will be present at the annual meeting of the Dutch Butterfly Conservation where this project will be presented as well. Citizen scientists that are interested will be sent a Dutch version of this deliverable with instructions. Suitable containers for sampling will be provided with the instructions.

Naturally samples should only be taken where and when the water can be reached without risk for the person taking the sample. We expect at least some water bodies to contain pesticides, this will however be strongly diluted and the concentration far below a level where it would be any risk to the citizen scientist.



Figure 2. An example of a dragonfly monitoring transect with four sections. All dragonfly species are counted for each section.

5. Chemical Analysis

The identification and measurement of complex organic compounds, such as pesticides, is highly specialised and requires techniques such as gas chromatography (GC) and high-performance liquid chromatography (HPLC). This is not feasible with citizen scientists. We identified possible partners that are able to do these analyses through our network and contacted a research group that uses these techniques and teaches students how to develop protocols for specific questions. The analysis of the samples will now be done at the University for Applied Sciences Leiden under the supervision of Dr. Peter Lindenburg. Several protocols for similar questions have been developed here in the past. Students will optimize the necessary techniques in collaboration with DBC and with advice from Prof Martina Vijver, an expert on pesticide exposure in aquatic ecosystems. Dr Peter Lindenburg is a biologist and biopharmaceutical scientist and has specialised in the measurement of low concentrations and quantities of organic compounds and currently works at Analytical BioSciences and Metabolomics at Leiden University and Metabolomics at the University for Applied Sciences Leiden. He will be the primary supervisor for the analysis, he and his team have a wealth of experience in the measurement of low concentrations of complex compounds through HPLC and GC.

6. Statistical Analysis

The concentrations of pesticides will likely not be independent from each other, as it is expected that certain pesticides frequently co-occur, and therefore we will apply a correspondence analysis. We will correlate the main axes of this analysis with the monitoring data on the dragonflies. This will give us insight into whether the occurrence of dragonflies, in both number, trend and species richness, is related to the exposure to pesticides. These results will be communicated to the citizen scientists through the newsletter of the monitoring scheme. The individual citizen scientists will also get the concentrations measured at their transect with an indication whether those levels are high or low.