Minimum detectable differences (MDD's) in mesocosm studies: The importance of pre-treatment management and appropriate sampling techniques for insecticides

ALEX BLAKEY, SEAMUS TAYLOR AND SARAH PRIESTLY

CAMBRIDGE ENVIRONMENTAL ASSESSMENTS, UK

Introduction

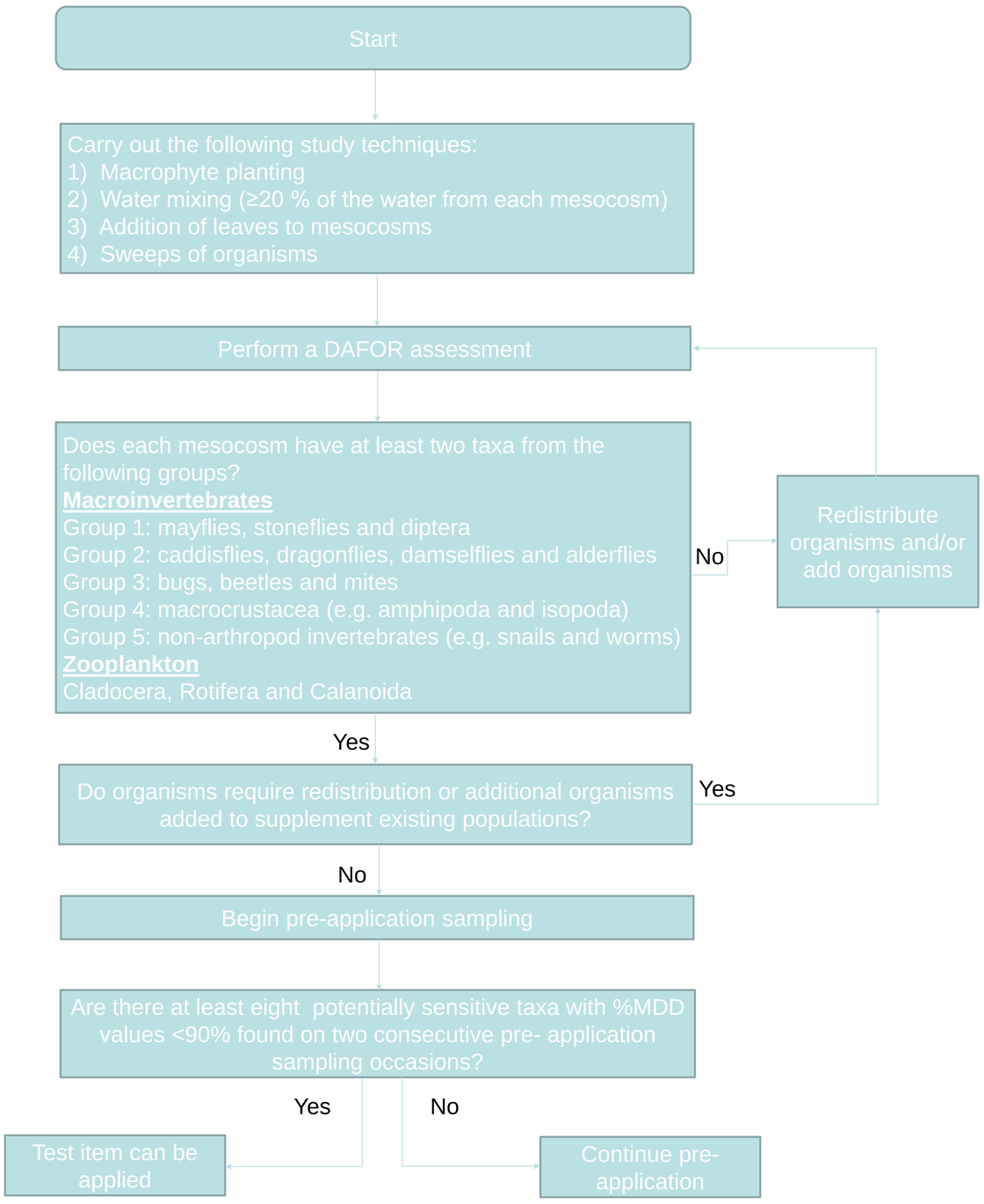
The Aquatic Guidance Document (EFSA, 2013) makes a number of recommendations for aquatic mesocosm studies including the reporting of Minimum Detectable Difference (MDD) values alongside No Observed Effect Concentrations (NOEC). In addition, it is recommended that each study contain at least eight different sensitive and vulnerable groups for which robust statistical evaluation is possible in order to be able to derive the Ecological Threshold Option (ETO) or the Ecological Recovery Option (ERO) –Regulatory Acceptable Concentrations. Here we will show the importance of study design focussing on pre treatment and in-life sampling techniques by drawing on recent mesocosm studies in order to produce robust data from studies with insecticides.

Pre- application set up of mesocosms

In order to derive robust endpoints it is important to ensure that each replicate mesocosm has diverse and sufficient populations prior to the application of an insecticide.

Here at Cambridge Environmental Assessments (CEA), we use a number of pre-application study design techniques when establishing replicate mesocosms to ensure that the mesocosms have similar and diverse populations of organisms. This is shown below in Figure 1.

Figure 1. Flow chart: Pre-application study design



Pre application MDD values

Here at CEA, to assess whether the mesocosms contain suitable populations of invertebrates, in order to allow the elucidation of the potential effects of a insecticide on the communities, we perform MDD analysis on our pre application data. We recommend at least eight taxa with MDD values <90%, preferably <70%, prior to application of the test item.

Using data from a recent mesocosm study we demonstrated that suitable populations of invertebrates were present with sufficient statistical confidence on back to back pre-application sampling days as shown in Table 1.

Table 1. Pre-application MDD values

Asellidae	Chaboridae	Non-arthropods	Cyclopoida	*Keretella* sp.	*Lepadella* sp.	*Nauplia* sp.	*Polyarthra* sp.
%MDD value	%MDD Value	%MDD Value	%MDD Value	%MDD Value	%MDD Value	%MDD Value	%MDD Value
25	65	72	71	70	51	15	59
23	47	76	75	72	61	17	83

In-situ bioassays

Where univoltine and multivoltine species are expected to be the sensitive organisms, in-situ bioassays can be used to collect additional quantitative information on potential impacts on populations. Here at CEA, we recommend introducing ≥10 organisms into a netted cage with portions of plant material and alder leaves as shown in Figure 2. Caged organisms should remain in-situ for 96 hours before being removed and the number surviving and dead organism counted to calculate the survival rate vs the control.

Figure 2. In-situ bioassays

Recommended in-life sampling techniques

To assess macroinvertebrate populations, we use colonisers (pebble baskets and *Elodea* traps) and sweep nets. Previous work conducted at CEA has shown that analysing sweep nets and colonisers as separate data sets results in higher MDD values for taxa that are not normally found in high numbers. As a result, we recommend pooling data for sweep nets and colonisers to form one robust data set for statistical analysis.

In addition, we recommend placing two emergent trapping vessels into each mesocosm to increase the surface area coverage. The larger surface area will increase the number of emergent insects caught which has been shown to decrease %MDD values by 5-25% (Brock *et al*., 2014).

Conclusions

In order to produce reliable endpoints from fresh water mesocosm studies with insecticides, we recommend the following:

- Mesocosms should be set up to contain diverse and sufficient populations of organisms.
- Eight potentially sensitive taxa should have %MDD values of <90% on two consecutive pre-application sampling occasions.
- In-situ bioassays should be used when univoltine or multivoltine are potentially a vulnerable taxa.
- Macroinvertebrate colonisers and sweep nets should be pooled to produce one data set.

References

[1] Brock, T. C. M., Hammers-Wirtz, M., Hommen, U., Preuss, T. G., Ratte, H-T., Roessink, I., Strauss, T. and Van den Brink, P. J., (2014). The minimum detectable difference (MDD) and the interpretation of treatment-related effects in experimental ecosystems. Environ Sci Pollut Res DOI 10.1007/s11356-014-3398-2.

[2] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Scientific Opinion - Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013; 11(7): 3290.