The Minimum Detectable Difference (1): Appropriate experimental design and endpoint selection for macrophytes in mesocosm studies

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INTRODUCTION

- The MDD (Minimum Significant Difference) value has been recommended by the European Food Safety Authority (EFSA) as a statistical robustness indicator [1]. It defines the lowest significant difference between the control and treatment that must exit to detect a statistically significant effect [2].
- Cambridge Environmental Assessment (CEA) has worked on improving mesocosm experimental design over time, going from flat bottomed mesocosms to more sophisticated sloped systems, reproducing a more realistic aquatic ecosystem.
- Our aim is to analyse how experimental design improvement and endpoint (effect parameter) selection can influence the amount of reliable data coming out of a study.

METHOD

A global comparison among studies differing in experimental design over time was undertaken by analysing the MDD values for each study and the corresponding effect parameters.

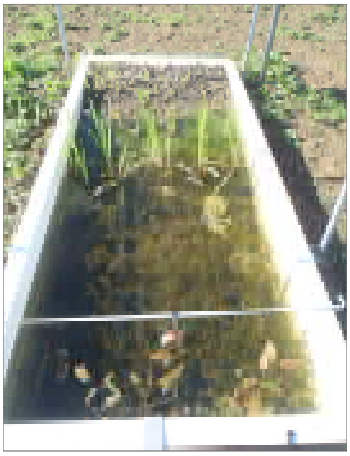

Figure 1. Sloped mesocosm. Study 4 , Control treatment on Day 14

4 herbicide studies were evaluated

Study 1: *Flat bottomed mesocosms*
- 9 plant species
- 20 measured effect parameters

Study 2: *Flat bottomed mesocosms*
- 10 plant species
- Plants set in pots at different depths
- 34 measured effect parameters

Study 3: *Sloped mesocosms*
- 10 plant species
- 46 measured effect parameters

Study 4: *Sloped mesocosms*
- 11 plant species introduced.
- 44 measured effect parameters

MDD calculation

- The MDDs were calculated using a two sided Dunnett's t-test. Items selected for analysis that relate to abundances of individual organisms were transformed onto the $\log_{10}$(abundance + 1) scale
- The %MDD (relative value) was used in this analysis

$$\%MDD = \frac{MDD}{\bar{x}_C}.100$$

$\bar{x}_c$= control mean

- The MDD values were characterised using the principles outlined by Brock *et al* [2] and the %MDD values were clustered into five classes following the Aquatic Guidance Document [1]
  - Class 0: >100%
  - Class I: 90-100%
  - Class II: 70-90%
  - Class III: 50-70%
  - Class IV: <50%
- The relative MDD class distribution (%) was then calculated for each selected effect parameter and the values calculated from each study were compared looking for trends

RESULTS

- Study designs and techniques have improved over time, with more healthy growing species included in the analysis giving a higher number of measurable effect parameters.
- Numbers of effect parameters giving reliable (MDD<100%) increased over time, with improved results in sloped mesocosms (Table 1).

Table 1. Number of effect parameters included in Category 1

Species	Study 1	Study 2	Study 3	Study 4
Elodea canadensis	3	-	6	4
Glyceria maxima	0	6	5	6
Hypuris vulgaris	0	3	5	5
Myriophyllum spicatum	2	0	5	4
Sarittaria sagittifolia	2	0	0	6
Potamogeton lucens	0	0	5	6
Ceratophyllum demersum	1	0	0	4
Veronica beccabunga	0	1	5	5
Ranunculus aquaticus	0	1	0	2
Total macrophytes	0	2	3	3
Total no. reliable parameters	8	13	34	40

- Additionally, an increase in the number of reliable endpoints giving % MDD values included in class III and IV (Figure 2) was observed with a greater proportion of Class IV endpoints in sloped mesocosms.

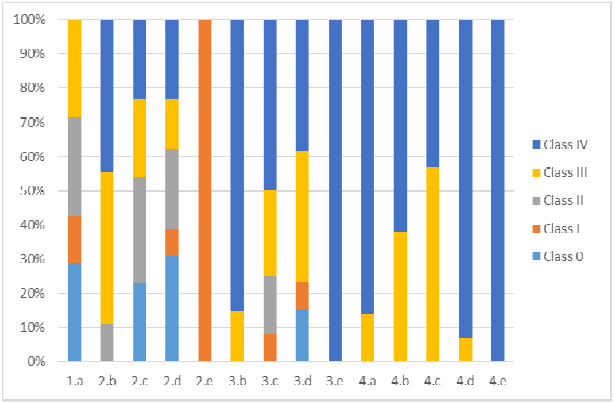

Figure 2. Relative class distribution (%) for selected parameters along the 4 studies. Where 1: Study 1; 2: Study 2; 3: Study 3; 4: Study 4 / a: *Sagittaria* stem length; b: *Glyceria* no. stems; c: *Hyppuris* no. stems; d: *Hyppuris* stem length; e: Total macrophyte dry weight; *f: Elodea* nodes (nodes/cm)

CONCLUSIONS & RECOMMENDATIONS

- Experimental design significantly influences the amount of reliable data generated in mesocosm studies
- The use of sloped mesocosms in herbicide studies seems to improve MDD values for macrophyte parameters
- Stem length, number of stems, nodes/cm or total macrophyyte dry weight could be reliable effect parameters
- More research on studies with MDD analysis is needed to provide more robust recommendations for good experimental design and measurement parameter selection

REFERENCES

[1] 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.
[2] 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.