PRACTICAL EXPERIENCE OF MDD ANALYSIS FOR MESOCOSM STUDIES: CHALLENGES FOR RELIABLE ENDPOINTS FROM TRICKY TAXA

Hanna Schuster, Fiona Joyce & Frances Pickering

Cambridge Environmental Assessments, Boxworth, UK; hanna.schuster@cea-res.co.uk

Cambridge Environmental Assessments

1. BACKGROUND

- The guidelines for the analysis of mesocosm studies for PPPs[1, 2] support the use of Minimal Detectable Difference (MDD) as part of the statistical analysis.
- This approach increases transparency and reliability of endpoints as it highlights the reliability of data or the lack of it.
- To comply with these still recent changes in the guidance, historic mesocosm studies have to be re-analysed using MDD analysis.

CEA has applied the new rules to a number of studies and the following findings can be highlighted:

- Interpretation of results within this guidance is straight-forward for high abundant taxa with a clear monotonous dose response.
- Taxa with insufficient data (Category 3) can be discarded as not meaningful based on high MDD values.
- Tricky taxa: those with sufficient data to be Category 1 or 2 but where the data quality varies throughout the study.

→ **Analysis of tricky taxa can be very subjective resulting in greatly varied endpoints from different parties, within and across organisations**

2. AIMS

Here we present examples of the practical approach that CEA have taken in assessing variable data, thereby highlighting the process of decision making and finding reliable endpoints for tricky taxa.

CAUSES FOR AND CHARACTERISTICS OF TRICKY TAXA

PROBLEMS ENCOUNTERED WITH HISTORIC MESOCOSM:

- Sampling time points:
  - No sampling directly before 8 weeks recovery deadline
  - No pre-app sampling
  - No sampling directly following dosing
- Taxonomic resolution too low
- Concentration range and spacing

SEASONALITY & ECOLOGY:

- Some species are only abundant at certain sampling occasions throughout the study
- Seasonal decline towards the end of the study
- Boom and bust taxon (Algae, Rotifers)
- Predator only abundant in low numbers (Odonata)

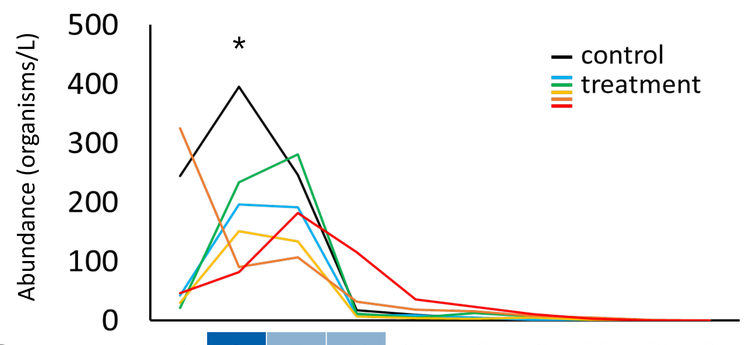
Inappropriate study design & seasonality → Low abundance & variability → High %MDDs → Endpoint uncertainty

OPEN QUESTIONS

How can we reduce uncertainty?
When do we include or exclude this data?
How do MDDs increase certainty?
How can subjectivity be reduced?
When is it relevant?

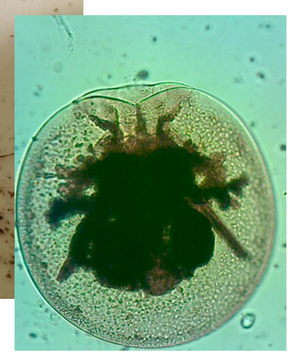
3. Tricky Taxon 1

- MDD category 2
- Statistical significant effect on day 3 at the two top concentrations (Williams' t-test)
- No reliable recovery demonstrated MDD >70% on day 7 and > 90%[2] on days 14 to 84



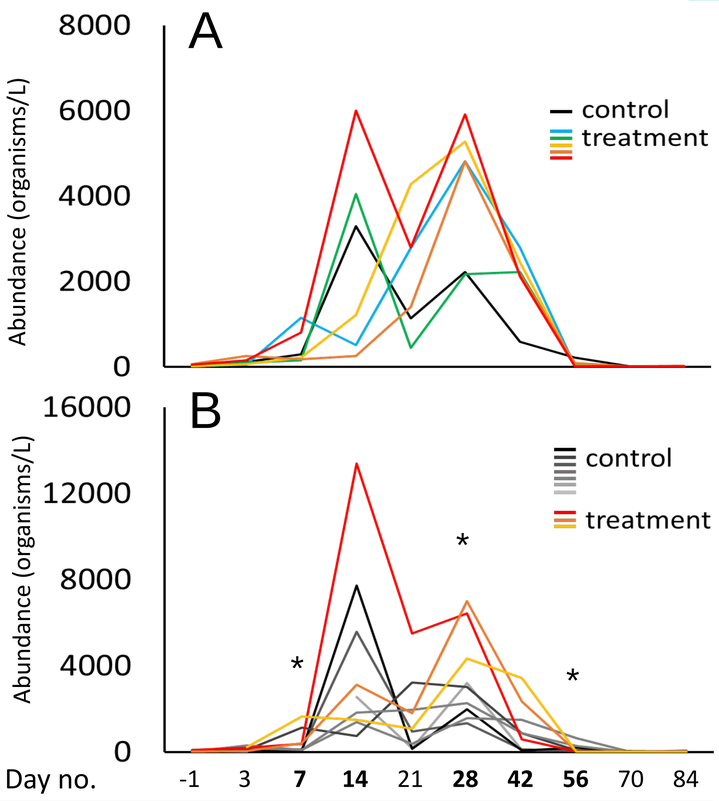
Day no.	-1	**3**	7	14	21	28	42	56	70	84
%MDD	92	**60**	77	100	98	122	201	245	600	678
NOEC (MDD Class)	6 (I)	**4- (III)**	6 (II)	6 (0)	6 (I)	6 (0)	6 (0)	6 (0)	6 (0)	6 (0)

Fig 1: Mean abundance of the control and five treatment levels. %MDDs and NOEC corresponding to each sampling occasion listed in the table above. Significant difference are highlighted in bold in the table and as * in the graph



4. Tricky Taxon 2

- MDD category 1 data with high variation
- Statistically significant effects on days 7, 14, 28 , 42 and 56 at all concentration (Dunnett's t-test)
- The effects observed are characterised by both increases and decreases
- No reliable recovery observed %MDD >100 on days 70 and 84



Day no.	-1	3	**7**	**14**	21	**28**	**42**	**56**	70	84
%MDD	115	81	**78**	**83**	75	**51**	**71**	**95**	101	304
NOEC (MDD Class)	6 (0)	6 (II)	**5+ (II)**	**4- (II)**	6 (II)	**3+ (III)**	**<2+ (II)**	**3- (I)**	6 (0)	6 (0)

Fig 2: A. Mean abundance of the control and five treatment levels. B. Abundances in the individual replicates of the control and the highest treatment. %MDDs and NOEC corresponding to each sampling occasion listed in the table above. Significant difference are highlighted in bold; * significant effect treatment 6.

5. HOLISTIC APPROACH

Effect classes assignment is based on four main basic pillars: Statistic, MDDs, Raw Data and Ecology

Considerations for tricky taxa 1

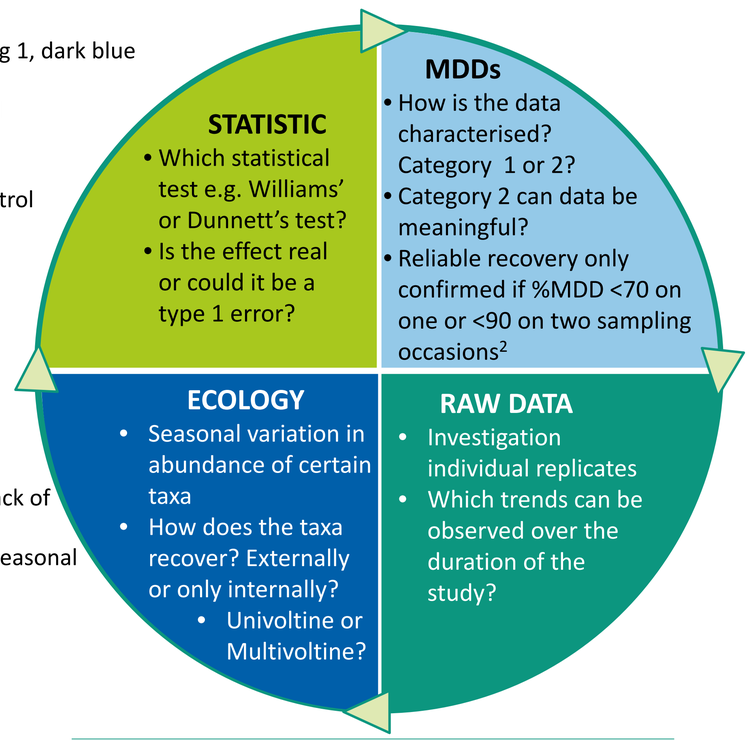
- Monotonous dose response relationship on day 3 → Williams' t-test used to analyse the effect (Fig 1, dark blue highlighted MDDs and NOEC).
- Category 2 data → meaningful as effect observed immediately following test item application and monotonous dose relationship observed.
- On day 7 all replicates of the top concentration (red line) are in the control range
- Pre-app abundances higher in the control than the treatment, but this was mainly due to one control replicate only. By day 3 abundances were high in all control replicates.
- High abundances at the start of the study but low and variable from day 14 onwards.
- MDDs too high to able to confirm recovery reliably[2] (MDDs and NOEC highlighted in lighter blue).
- This is due to seasonal decline from day 14 onwards.

→ Effect class: 2-4B at concentration 5 and 6

Considerations for tricky taxa 2

- Effects on days 14, 28 and 42 were discarded at all but the top concentration (red line) due to a lack of clear monotonous dose relationship as highlighted in turquoise (Fig 2 A).
- The effects on day 7 and 56 at the top concentration (red line) are likely to be a result of natural seasonal variation and were therefore discounted.
- Only the effect on day 28 is considered relevant as all replicates are outside the control range.
- High %MDDs following the effect on day 28 do not allow recovery to be reliably confirmed[2].

→ Effect class: 2-4B at concentration 6 only



6. CONCLUSIONS & RECOMMENDATIONS

- MDD analysis can increase the transparency and reliability of mesocosm results.
- The characterisation of data into Category 1 and 2 is a helpful tool to assess the relevance of large data sets.
- However, some taxa can be characterised as Category 1, for which further review of the raw data and ecology is a necessity.
- At CEA we strongly recommend using all available information as not all statistically significant effects are biologically relevant.
- The addition of MDD analysis has had a large impact on the acceptance of recovery following an effect during the review of historic mesocosm studies potentially leading to lower endpoints.
- Therefore, we recommend further clarification of recovery assessments using MDDs by EFSA in the EFSA guideline[1].

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. (2015). The minimum detectable difference (MDD) and the interpretation of treatment-related effects in experimental ecosystems. Environ Sci Pollut Res