Selecting and testing ecologically relevant macrophytes and endpoints in mesocosm studies

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CEA
CAMBRIDGE ENVIRONMENTAL ASSESSMENTS

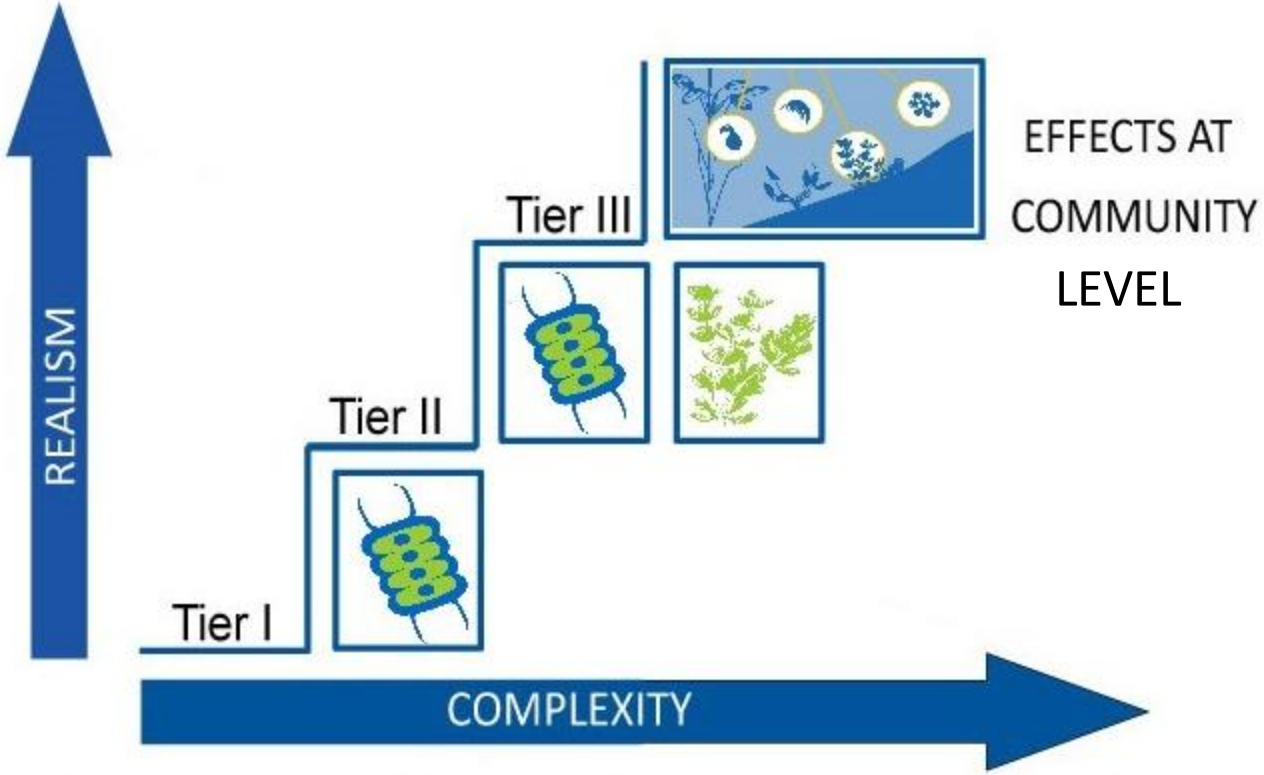
Outline

- Why are macrophytes important?
- Current risk assessment and protection goals for macrophytes
- Regulatory acceptable mesocosm studies
- What is Representative and Relevant?
- Selecting macrophytes for mesocosm studies
- Endpoint selection for macrophyte mesocosm studies
- CEA sloped mesocosms
- Recommendations for mesocosms

Why are Macrophytes Important?

- Play a vital role in aquatic ecosystems by:
- Providing refuge and shelter for other organisms.
- Providing a food source.
- Influencing water quality and carbon and nutrient cycles.
- Ensuring a biodiverse and resilient ecosystem.

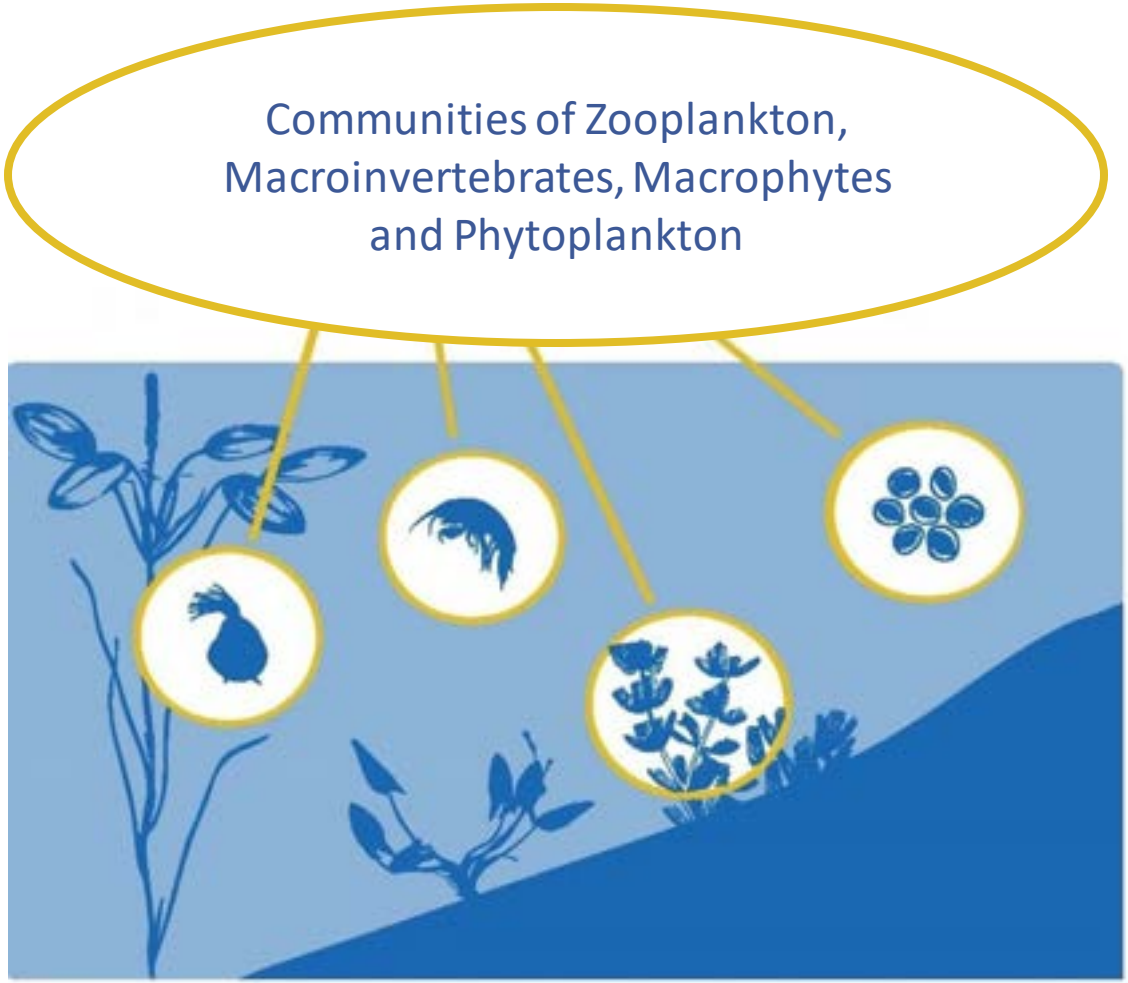
Current Risk Assessment and Protection Goals



Tier I studies: Core acute/chronic data (standard species and exposure)
Tier II studies: Acute/chronic data from additional species or refined exposure
Tier III studies: Population and community level experiments (e.g. mesocosms)

Regulatory acceptable mesocosm studies

- Should reflect the communities found in the natural environment.
- EFSA (2013) recommends 8 sensitive/vulnerable taxa with acceptable minimum detectable difference (MDD) values.

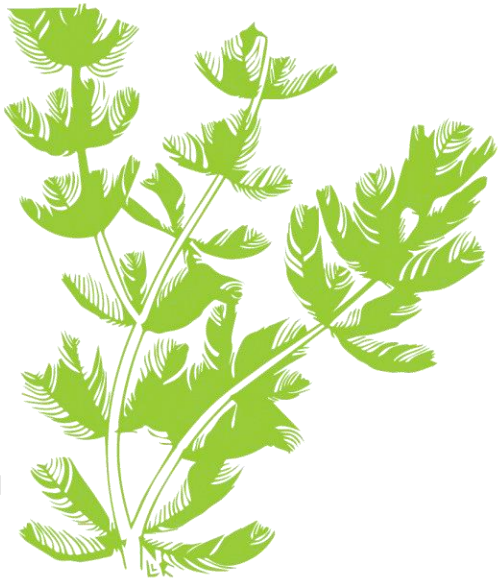


What is Representative and Relevant?

- **How can one test system represent all waterbodies, locations and species?**
- Very little guidance on what species should be included in mesocosm studies.
- Limited formal guidance for evaluating the relevance of a study.
- Expert judgement required to design higher tier studies.
- What does ecologically relevant mean?

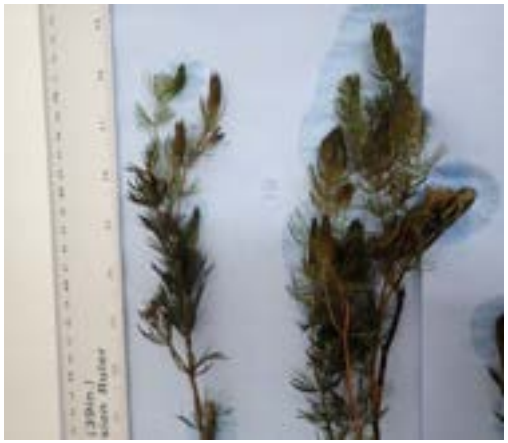
Macrophyte Selection Criteria

- Use some standard laboratory test species.
- Sensitive species.
- Perennial (emergent) macrophytes that are likely to suffer herbicide stress.
- Consider the mode of action of the test substance.
- Variety – include different classes and morphological characteristics (monocots and dicots, floating and rooted, submerged and emergent).
- Consider the ecology and distribution of each macrophyte.
- Consider what data/endpoints will be collected.
- Include oxygenating macrophytes.



Macrophyte Selection Examples

Myriophyllum spicatim **(Water milfoil)**	
• Standard test species • Submerged • Dicot	• Invasive submerged aquatic weed • Widespread and common



Glyceria maxima **(Reed sweet grass)**	
• Standard test currently being developed • Emergent • Monocot	• Aquatic, perennial grass • Native to temperate Europe and Asia • spreads vigorously from rhizomes and seedlings

Macrophyte Selection Examples

Hippuris vulgaris **(Common mare's tail)**	
• Perennial emergent macrophyte • Dicot	• Widespread common aquatic plant in Europe, Asia and North America

Sagittitaria sagitifolia **(Native Arrowhead)**	
• Perennial emergent macrophyte • Monocot	• Flowers July to August • Grows in shallow water • Common and widespread • Native to wetlands of Europe

Macrophyte Selection Examples

Potamogetum natans **(Broad-leaved pond weed)**	
• Floating leaves • Monocot	• Wide ecological tolerance • Leaf and flower stalks grow from rhizomes • Grows in deep water

Veronica beccabunga **(Brooklime)**	
• Emergent Perennial bulb • Dicot	• Occurs in most types of wetland and riparian habitats • Grows in shallow water • Flowers May to August

Macrophyte Selection Examples

Elodea canadensis **(Canadian pondweed)**	
• Rooted, submerged • Monocot	• Oxygenating plant • Perennial with a broad habitat range • Fast growing

Ceratophyllum demersum **(Hornwort)**	
• Unrooted, submerged • Dicot	• Oxygenating plant • Wide ecological tolerance • Reproduction by vegetative fragmentation

Macrophyte endpoints

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Height Measurements

Counts of Nodes

Counts of Flowers

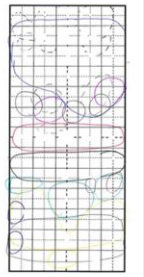
Counts of Leaves

Number of Stems

chlorosis/necrosis

Weekly Biomass

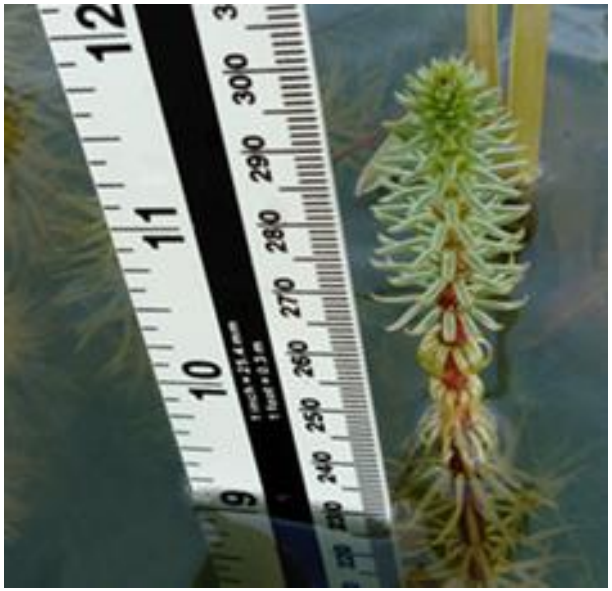
Aerial coverage



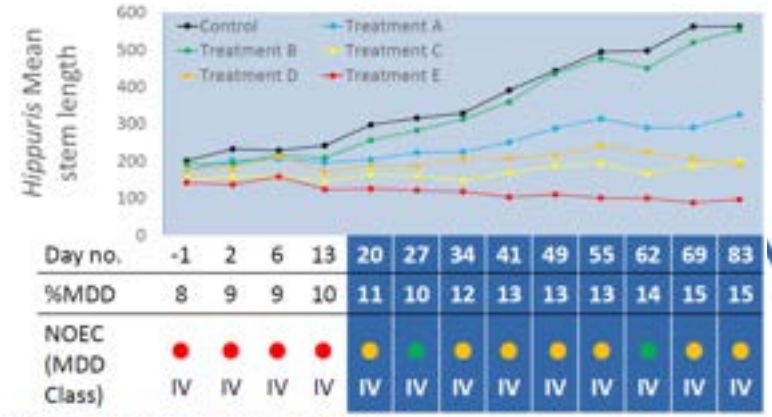
Wet and Dry Weight

Multiple Plant Health and Growth Measures

- Choose appropriate measurements and endpoints tailored to each macrophyte.
- Multiple endpoints for each species, provides lots of data on the effects of the test item on the community and more evidence that endpoints are robust and reliable.
- Less variability/lower MDDs and increased statistical power.

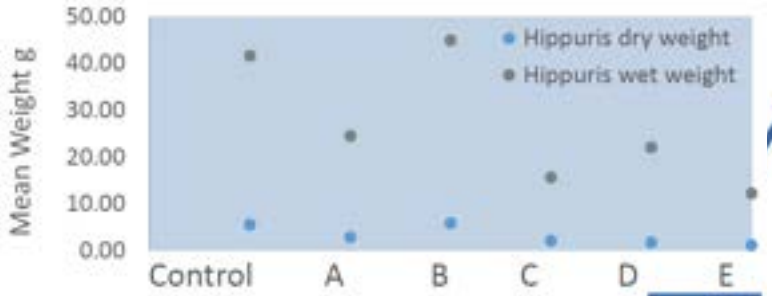


Case Study: Hippuris Vulgaris



Day no.	-1	2	6	13	20	27	34	41	49	55	62	69	83
%MDD	8	9	9	10	11	10	12	13	13	13	14	15	15
NOEC (MDD Class)	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV

Figure 1: **Mean Stem length**

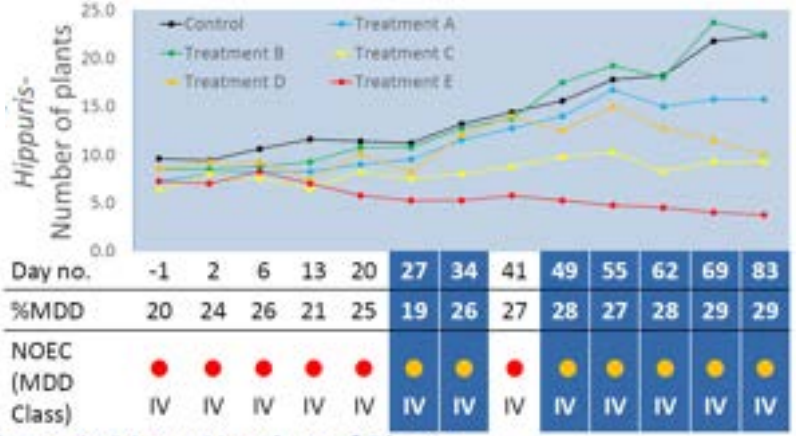


wet weight.	Day83
%MDD	49
NOEC (MDD Class)	E (IV)

dry weight.	Day 83
%MDD	43
NOEC (MDD Class)	D (IV)

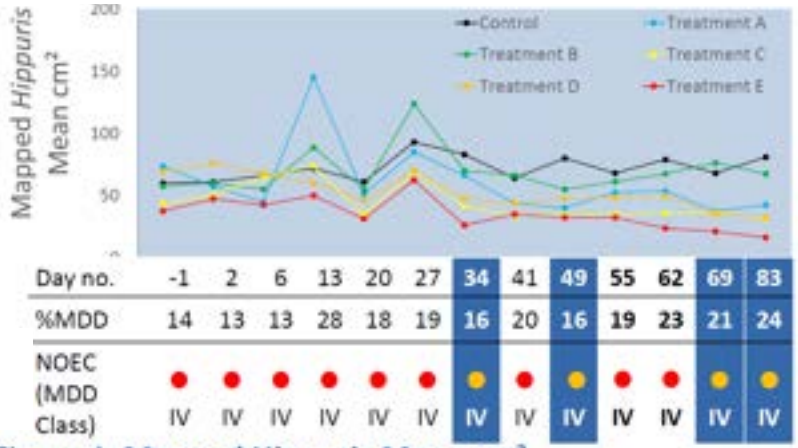
Figure 3: **Wet and Dry Weight**

Overall NOEC



Day no.	-1	2	6	13	20	27	34	41	49	55	62	69	83
%MDD	20	24	26	21	25	19	26	27	28	27	28	29	29
NOEC (MDD Class)	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV

Figure 2: **Mean Number of Plants**



Day no.	-1	2	6	13	20	27	34	41	49	55	62	69	83
%MDD	14	13	13	28	18	19	16	20	16	19	23	21	24
NOEC (MDD Class)	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV

Figure 4: **Mapped Hippuris Mean cm²**

CEA Sloped Mesocosms

- Sloped mesocosm with a gradient allows macrophytes to be planted at different depths.
- Macrophytes tested have optimal growing conditions with varying light levels and niches for macrophytes planted at different depths.
- Allows for species interactions, replicating true community effects and a more realistic assessment of effects

Planting Depths

Species	Planting depth (cm)
Veronica beccabunga	0 - 10
Myotis scorpioides	10 - 20
Sagittaria sagittifolia	20 - 30
Hippuris vulgaris	20 - 30
Glyceria maxima	30 - 40
Myriophyllum spicatum	40 - 50
Elodea Canadensis	50
Potamogeton natans	50

Recommendations

- Using a sloped mesocosm design creates the optimal growing conditions for a wide variety of macrophytes.

- A variety of macrophytes should be tested to ensure the risk assessment is comprehensive and to ensure that macrophyte protection goals are achieved.

- The endpoints selected should be comprehensive enough to determine the effects of the test item on individual species and communities as a whole.

Conclusions

Mesocosm studies can be an excellent tool in herbicide risk assessments if:

- Mesocosm studies are designed to be **ecologically relevant and representative**.
- Careful consideration is given to the selection of macrophytes and endpoints to meet EFSA requirements for 8 **sensitive/vulnerable** taxa with acceptable MDD values.
- Studies are designed to derive more **reliable** and **robust** community level endpoints.

Any questions?

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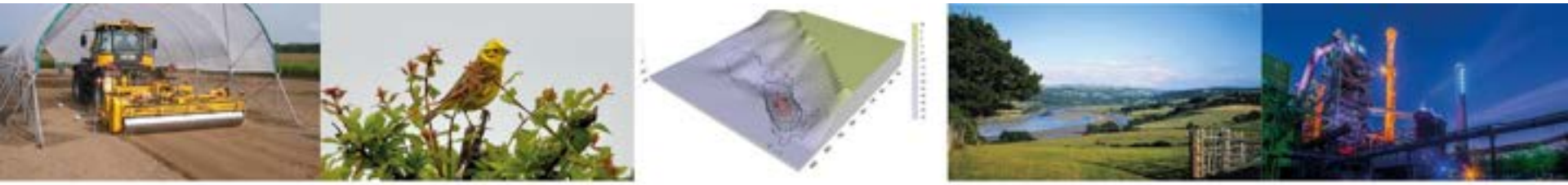

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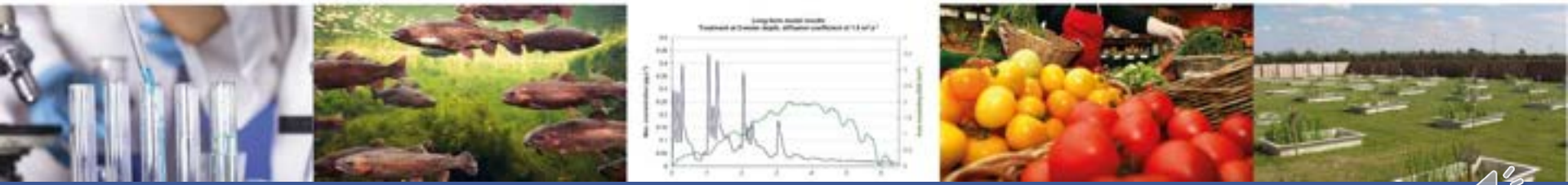
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