NEW DEVELOPMENTS IN MESOCOSM TESTING WITH HERBICIDES: RECENT EXPERIENCE AND RECOMMENDATIONS

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Abstract

A number of new developments in mesocosm testing have taken place since the most recent revisions to guidance for these complex studies (e.g. HARAP, 1998 & CLASSIC, 2001). Much of the progress in this area since this guidance was produced has been in the design and conduct of freshwater mesocosm studies to evaluate the toxicity of herbicides on algae, periphyton and macrophytes. We aim to highlight some of the developments in this area using examples drawn from recent state of the art mesocosm studies. We will provide recommendations for the evaluation of effects on plants, algae and periphyton by drawing on our experience in this area. It is hoped that this information will advance mesocosm study design and offer advice to those considering conducting mesocosm studies with herbicides whilst stimulating discussion in this developing area of higher tier aquatic ecotoxicology.

Macrophyte health measurements

We identified a need to provide a way to collect qualitative and quantitative data for a robust assessment of macrophyte health so we have attempted to design a holistic system that tries to capture differences related to specific functional groups.

For qualitative assessments, macrophytes are given a score based on a visual evaluation of their appearance, coverage and structure against a set of prescribed criteria which are selected based on specific morphological traits for individual plant taxa. An example of the scoring matrix for *Elodea* sp. is presented in Table 1. The cumulative score is then analysed statistically for each replicate to provide an assessment of effects.

Appearance (A)	Coverage (C)	Structure (S)
3 = healthy (uniform, green and dense foliage)	3 = uniform mat	3 = large multi-branched, single piece
2 = average (green/brown with patchy foliage)	2 = patchy coverage & sediment visible	2 = mixture of medium sized multi branched and multiple small pieces
1 = poor (brown leaves, brittle stems)	1 = sparse coverage	1 = mostly multiple small pieces

Table 1. Example scoring matrix for Elodea sp.

Qualitative scoring assessments are then supported with quantitative assessments of plant growth e.g. the total number of *Sparganium* sp. leaves per occasion as shown in Figure 1.

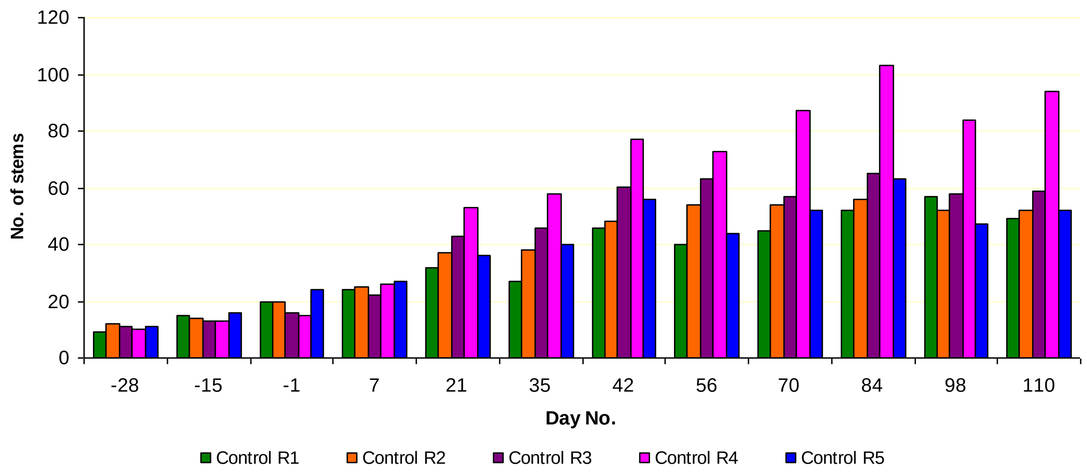

Figure 1. Sparganium sp. *stem numbers over time*

Community vs. population level effects

Potted plants provide a useful and robust analysis of effects on discrete populations however, community effects can be missed when only potted populations are exposed to test items in mesocosm studies. The use of 'free living' plants in addition to contained populations facilitates an assessment of community responses to treatment in addition to more specific population level effects.

Population effects are monitored using a series of quantitative and qualitative methods such as macrophyte health assessments whereas community responses are assessed using macrophyte mapping and harvesting of the total biomass at the end of the test. Both community and population responses are then statistically assessed to derive community and population endpoints.

Un-rooted macrophytes

Un-rooted macrophytes e.g. *Lemna* sp. are often difficult to monitor in mesocosm studies due to the way they can disperse and become entangled with other plants and algae. As a result, floating rings (Figure 2) designed to contain their growth were employed in a mesocosm study treated with a herbicide. However, *Lemna* growth was poor which was attributed to low levels of dissolved nutrients.

To negotiate this problem, laboratory bioassays based on an established test guideline (OECD 221) can be employed alongside mesocosm studies using water collected from treated mesocosms and spiked with nutrients. However, to avoid degradation of test item residues, samples of the mesocosm water should be sterilised with membrane filtration instead of autoclaving.

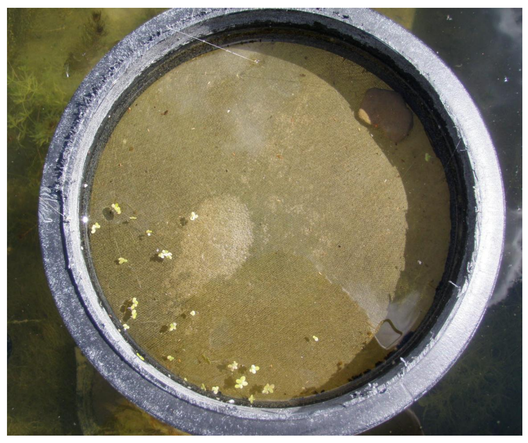

Figure 2. Floating Lemna ring

Naturally colonising plants

Plant health assessments and destructive sampling (Figure 3) should take account of the entire macrophyte community including naturally colonising taxa that were not introduced at the start of the study. However statistical analysis of non-introduced taxa is not recommended without careful examination of the data due to the inherent variability within these populations.

Other recommendations

Initial total biomass should be low enough to ensure effects on growth can be evaluated.

NOEAECs should not be derived from terminal biomass estimates as they do not provide a comparative assessment of recovery within the normal timescale for a mesocosm study.

Plant and algal health assessments should be used alongside sensitive functional endpoints such as dissolved oxygen production.

Figure 3. Destructive sampling

Photographical evidence should be used to support macrophyte health assessments as they provide a powerful tool to support qualitative evaluations.

Reference

OECD 221 (2006), OECD guidelines for the testing of chemicals. *Lemna* growth inhibition test.