# Floating leaves of *Potamogeton natans*: A new method to evaluate the development of macrophytes in pond mesocosms


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## Introduction

In mesocosm studies, the investigation of effects on macrophytes is often limited to final harvest and biomass determination. In the course of an experiment, the development of macrophytes which, for example, have been treated with herbicides can only be studied by surveying and mapping. For that reason, generating "hard data" is often difficult.

In the course of an indoor mesocosm pond study on the effects of a herbicide (Fig. 1), both number and area of floating leaves of *Potamogeton natans* were determined employing image analysis. The question was whether this time saving approach yields reliable results for number and area of floating leaves, which can be used as both intermediate and final endpoint.


**Fig. 1. Photos taken for the determination of the endpoint floating leaves in a herbicide effect study on day 50 after single application; 0 = control, 5 = 5 µg/L, 80 = 80 µg/L**

## Material and Methods

- Data were generated by means of image analysis (analySIS®) based on fortnightly photos (CANON S 40, 4 megapixel) taken at an angle of 30° from the water surface of the same side of the 3 controls and the 5 ponds which had been contaminated once with a herbicide at the start of the experiment (Fig 2).
- Counts included all leaves with more than 50% of green surface and were repeated by the same and by up to 4 different counters in order to check for precision and accuracy.
- Prior to area determination (pixel coverage over total pixel ratio) the photos were adjusted for perspective by means of GIMP® software (Fig. 3), treated with colour filters employing Microsoft Photo Editor® (Fig. 4) and subjected to colour detection with the analySIS® software (Fig. 5).
- The results were compared to the endpoints' wet weight and ash-free dry weight which had been determined at the end of the experiment.

**Fig. 2. Original photo of the water surface of an indoor pond taken at an angle of 30°**

**Fig. 3. Photo of Fig. 2 perspective adjusted with GIMP® software**

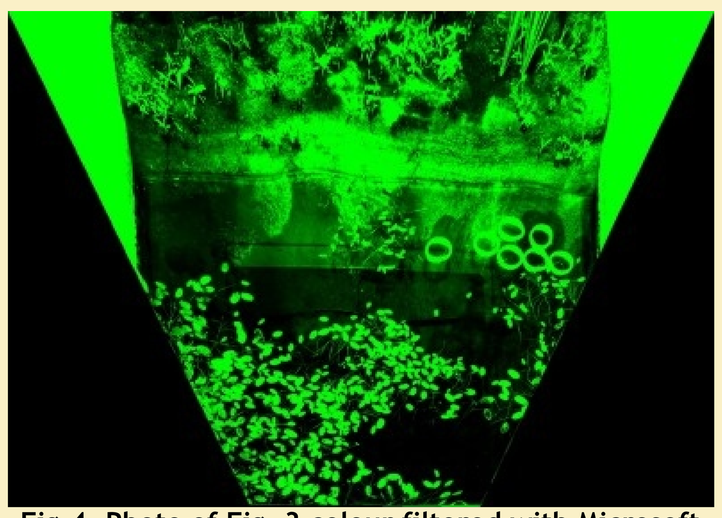

**Fig.4. Photo of Fig. 3 colour filtered with Microsoft Photo Editor®**

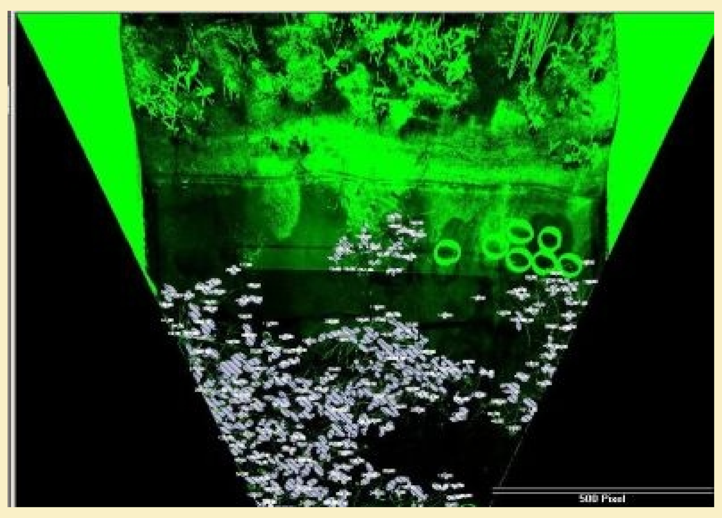

**Fig. 5. Photo of Fig. 4 after defining the 'regions of interest' and passage through the filters of analySIS®**

## Results

- For the endpoint 'number of leaves', variance introduced by the counters was negligible. The resulting patterns in the concentration effect relation were identical, since the differences were the same for each pond and mostly resulted from the subjective classification of the '50% green criterion' (Fig. 6)

- The possibility of introducing error into the leaf area measurements was greatest in setting of both colour filter with the Photo Editor and threshold for colour detection with the analySIS programme. However, both number and area of floating leaves proved to be reproducible and yielded easy to measure endpoints for observing temporal and pesticide related changes in standing stocks if these procedures were clearly defined prior to analysis and identically applied on all photos. This increase in precision is outweighed by a loss of accuracy, if the photos are not taken under identical light conditions and coordinates.

- Z-transformed data ((value - mean) / SD ) obtained with the 3 methods (area of leaves, number of leaves, total weight, Fig. 7) yielded similar results (Wilcoxon, RP = 18 resp. 14 > R(8, 0,05) = 4). However, the fit was greatest (RP = 18) between leaf area and biomass. Area detection can be considered the most reasonable endpoint for herbicide effects since

1. counts of leaf number do not regard the size of the leaves (many small leaves in pond 2 and 6 and fewer and larger leaves in pond 8, Fig 7) and

2. Biomass falsely includes dying off vegetation (pond 2, 3 and 6).

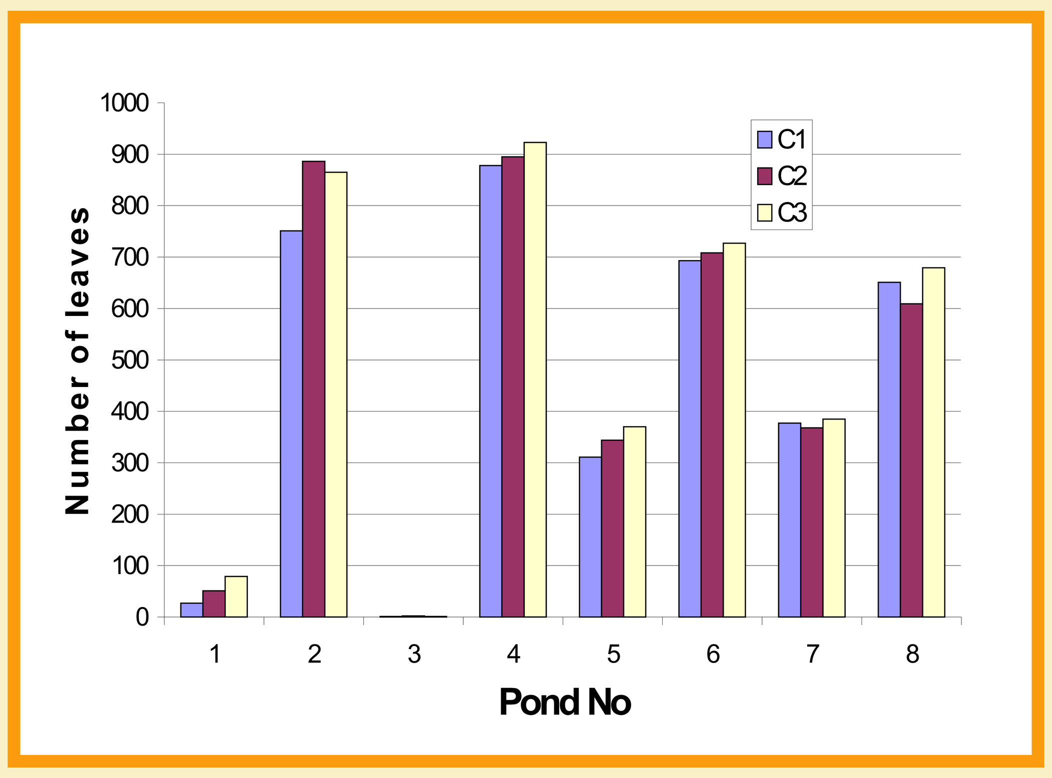


**Fig. 6. Repeated counts of the floating leaves of *Potamogeton natans* in the 8 differently contaminated indoor ponds on day 110 of the herbicide effect study (C1-C3 = different counters)**

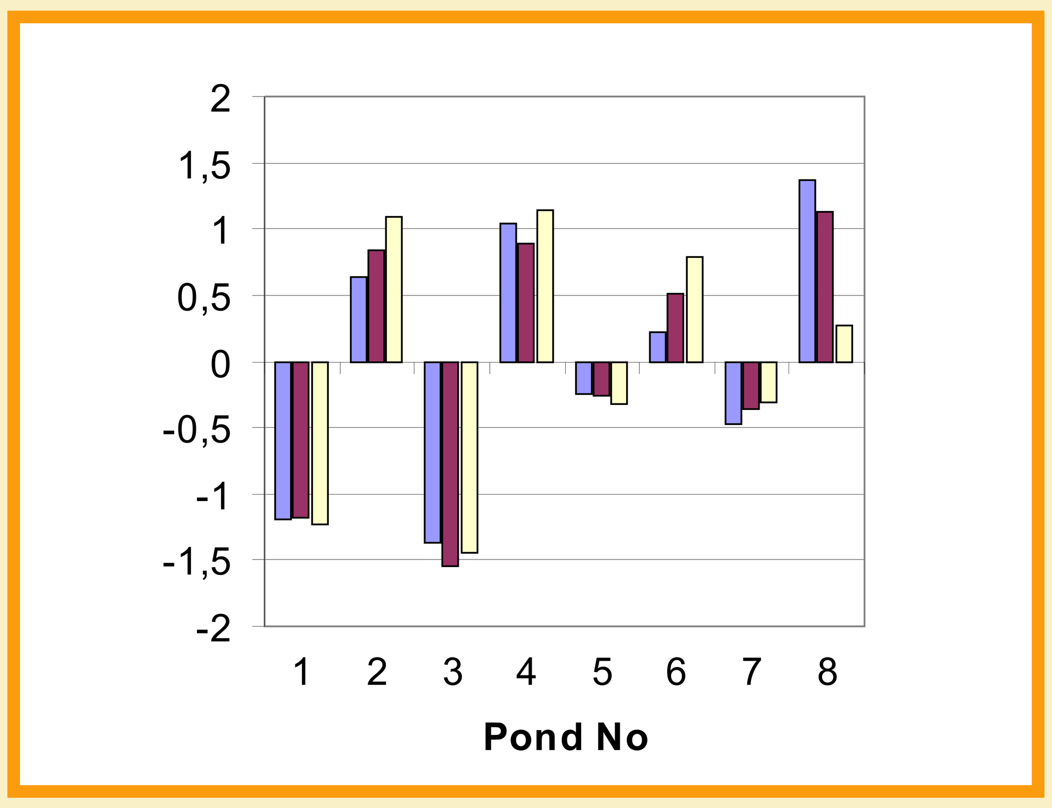


**Fig. 7. Z-transformed data ((value - mean) / SD) obtained with the 3 methods (area of leaves = blue, total weight = brown, number of leaves = yellow)**

## Conclusions

'Area of floating leaves' of *Potamogeton natans* is a perfect endpoint in freshwater mesocosm studies on the effects of herbicides. It is

- sensitive,
- easy to measure,
- yields more reasonable results as compared to biomass data or leaf counts and
- cannot only be used for final endpoint determination but also for intermediate evaluation in long-term experiments.

In this herbicide study, the $EC_{50}$ for *Potamogeton natans* calculated as time weighted average (TWA) based on wet weight was 44 µg/L whereas it was only 28 µg/L based on the measurements of the area of floating leaves.

In future studies, photos will by taken in a right-angle and centred position by means of a special construction and corrected for lens aberration in order to cover the entire water surface of the ponds and to reduce the error introduced by the adjustment of the perspective and light conditions.

The method may also be employed in the field if defined circular floats on the water surface can be used for precise perspective correction of the photos with GIMP®.

**Fig. 8. The artificial pond and stream mesocosm system - FSA - of the German Federal Environmental Agency**


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**Fig. 9: Labor-intensive harvest of macrophytes**