

# Experimental studies to provide long-term data sets for testing population models for *Lemna* sp. and *Myriophyllum spicatum*

Gertie H.P. Arts<sup>1</sup>, U. Hommen<sup>2</sup>, E. Bruns<sup>3</sup>, S. Heine<sup>3</sup>, A. Solga<sup>3</sup>, S. Taylor<sup>4</sup>

<sup>1</sup>Wageningen University and Research, Environmental Risk assessment, Droevendaalsesteeg 3, 6700 AA Wageningen, The Netherlands; <sup>2</sup>Fraunhofer Institute for Molecular Biology and Applied Ecology, Germany;

<sup>3</sup>Bayer AG, Germany; <sup>4</sup>Adama Agricultural Solutions Ltd.

E-mail contact: [Gertie.Arts@wur.nl](mailto:Gertie.Arts@wur.nl)

---

## 1. Introduction

For the analysis and prediction of the effects of diverse and dynamic exposure profiles of plant protection products on aquatic organisms, TK-TD (toxicokinetic- toxicodynamic) and population models are considered a promising tool in the higher tier risk assessment [1]. For two species of aquatic macrophytes, *Lemna* sp. and *Myriophyllum spicatum*, such models have already been developed [2,3]. The potential use of such models to address the variety of exposure profiles predicted e.g. by the FOCUS surface water models, has been shown during the MODELINK workshop [4]. For the calibration and validation of the population dynamic part of these models, available data from existing mesocosm studies are often relatively short (up to a few months). Moreover, these studies are usually not designed to provide data sets to test macrophyte models. Therefore, an experimental study was undertaken to provide long-term data sets of seasonal dynamics of *Lemna* sp. (expected to be primarily *Lemna minor*) and *Myriophyllum spicatum*. The aim was to generate a dataset that covers the seasonal dynamics of both macrophytes including periods of abundance at the carrying capacity of the experimental systems followed by an expected decrease of abundance and biomass later in the season in autumn and in winter. These data sets should allow testing and refinement of population models for both species for application in the risk assessment of plant protection products and other stressors.

## 2. Materials and methods

The study is carried out from spring/early summer 2017 until spring/early summer 2019 (2 winters included) under realistic environmental conditions in terms of water and sediment quality, weather, temperature and photosynthetic radiation. The conditions mimic the temperate climate in the Atlantic-subatlantic central zone of Europe. The nutrient levels are intended to prevent growth limitation. The growth of *Myriophyllum spicatum* is monitored in a large number of pond baskets (Fig. 1, left) installed in an experimental ditch (Fig 1, middle), while the *Lemna* sp. populations are studied in a set of microcosms (Fig. 1, right). The monitoring of biomass (fresh and dry weight) and shoot length or frond numbers is carried out weekly, biweekly or monthly, depending on the season (i.e. lower sampling frequency in winter and higher frequency during peak growth or until the water surface is covered). Apart from the standing crop, seasonal dynamics of growth rates of shoots or fronds over a time frame of 1 month (*Myriophyllum* shoots) or 1 week (*Lemna* fronds) are assessed by regularly introducing new plants in empty baskets respectively microcosms ('recovery tests'). In addition, relevant environmental parameters, e.g. water and air temperature, global radiation, photosynthetic active radiation (PAR), nutrient concentrations, pH and sediment quality are regularly measured.

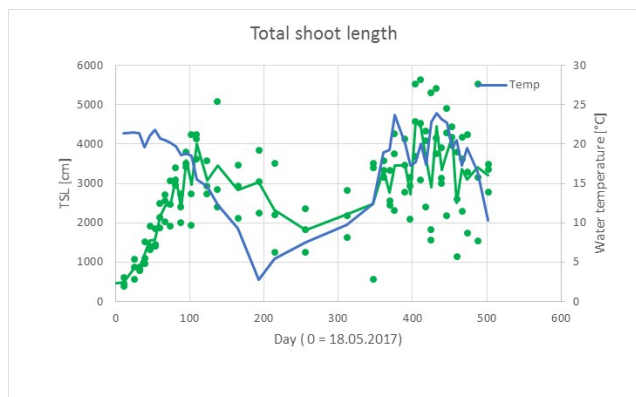
## 3. Results and discussion

### 3.1. Results of *Myriophyllum spicatum* monitoring

We observe a high variability in *Myriophyllum* total length and biomass among the baskets, which results in a strong variability of growth rates over time relative to the previous sampling date. Growth rates relative to time 0, which is the start of the monitoring study, show a fast increase in spring and decrease in autumn and thus reflect the seasonal dynamics over time. The growth rates of *Myriophyllum* shoots in recovery baskets are higher than those in the normal baskets and similarly reflect a seasonal. However, from October on, the shoots in the recovery baskets hardly develop roots. As a consequence, they do not anchor in the sediment and growth is hampered or prevented resulting in a loss of shoots.



**Figure 1:** *Myriophyllum* basket (left), ditch filled with baskets of *Myriophyllum spicatum* shoots (middle) and *Lemna* sp. microcosms (right).



**Figure 2:** Total shoot length of *Myriophyllum spicatum* as a function of temperature (average CV for 3 baskets for all data = 26 %). The green line represents the average of the 3 baskets sampled at each sampling date.

### 3.2. Results of *Lemna* sp. monitoring

The aim of the *Lemna* experiment was to mimic a growth curve under realistic favourable environmental conditions, starting from 100 fronds, subsequent density depending growth of *Lemna* sp up to full coverage and followed by a decline in autumn and winter. The main experiment was started in 2018, re-started afterwards and re-started in 2019 again. This was caused by a number of factors: 1. Competition with other primary producers such as phytoplankton (in year 1) and Floating Algae Beds (in year 2); 2. Disturbing birds and an invasion of the beetle *Phyllopertha horticola*. First results show extreme variability among the destructively (weekly to monthly) sampled microcosms. Full coverage of the microcosms however, was reached in late summer / autumn in both years. The recovery microcosms showed less variability and also temperature dependence.

## 4. Conclusions

A high variability in macrophyte growth was observed between replicates over time in both the *Lemna* sp. and the *M. spicatum* experiments. The data analysis that will be performed over the next months, will focus on answering the question which variables, that have been measured, and statistical parameters can be related to this variation. The recovery experiments and the permanently monitored microcosms will probably deliver the best information to the modellers. Untill spring 2019, the datasets will be completed and methods and results will be summarized for the *Lemna* sp. and *Myriophyllum spicatum* experiments 2017 – 2018.

## 5. Acknowledgements

This experimental study is a 3-year study and is equally financially supported by the TKI programme of the Dutch Minister of Economic Affairs and the European Crop Protection Association. Many thanks to the experimental team of Wageningen Environmental Research (Jasper van Smeden, Marieke Wolters, Arriëne Matser and Dick Belgers).

## 6. References

- [1] EFSA, 2018. Scientific Opinion on the state of the art of Toxicokinetic/Toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms
- [2] Schmitt, W., E. Bruns, M. Dollinger, P. Sowig, 2013. Mechanistic TK/TD-model simulating the effect of growth inhibitors on Lemna Populations. *Ecological Modelling* 255: 1– 10.
- [3] Heine, S., Schmitt, W., Schaffer, A., Gorlitz, G., Buresova, H., Arts, G., Preuss, T.G. 2015. Mechanistic modelling of toxicokinetic processes with *Myriophyllum*. *Chemosphere* 120: 292 - 298.
- [4] Hommen, U., Schmitt, W., Heine, S., T.C.M. Brock, S. Duquesne, P. Manson, G. Meregalli, H. Ochoa-Acuña, P. van Vliet and G.H.P. Arts, 2016. How TK-TD and population models for aquatic macrophytes could support the risk assessment for plant protection products. *Integrated Environmental Assessment and Management* 12 (1): 82 - 95. DOI: 10.1002/ieam.1715.