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Seasonal dynamics of the standard test species *Lemna* sp. in outdoor microcosms

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Abstract

Lemna L. sp. is a free-floating aquatic macrophyte that plays a key role as a standard test species in aquatic risk assessment for herbicides and other contaminants. Population modeling can be used to extrapolate from laboratory to field conditions. However, there are insufficient data on longer-term seasonal dynamics of this species to evaluate such models. Therefore, several long-term growth experiments were conducted in outdoor microcosms (surface area 0.174 m^2). Monitoring parameters included biomass, frond numbers, water parameters, and weather data. Three different datasets were generated: frond numbers and biomass from weekly to monthly destructively sampled microcosms; a year-round dataset of frond numbers from five continuously monitored microcosms; and seasonal growth rates without the effect of density dependence over 1–2 weeks in freshly inoculated microcosms. *Lemna* sp. reached a maximum of approximately 500 000 fronds m^{-2} and 190 g dry weight m^{-2}. During the first winter, the microcosms were covered by ice for approximately four weeks, and *Lemna* sp. populations collapsed. The second winter was warmer, without any ice cover, and *Lemna* sp. populations maintained high abundance throughout the winter. Dry weight per frond was not constant throughout the year but was highest in autumn and winter. Growth rates without density dependence under outdoor environmental conditions reached 0.29 day^{-1} for frond number, 0.43 day^{-1} for fresh weight, and 0.39 day^{-1} for dry weight. In linear regressions, these growth rates were best explained by water temperature. For the populations continuously monitored throughout a year, the nitrogen-to-phosphorus ratio best explained the growth rate of frond numbers. This study yielded a relevant dataset for testing and refining *Lemna* population models used in chemical risk assessment as well as for managing ecosystems and combating the effects of eutrophication. *Integr Environ Assess Manag* 2024;00:1–14. © 2024 The Authors. *Integrated Environmental Assessment and Management* published by Wiley Periodicals LLC on behalf of Society of Environmental Toxicology & Chemistry (SETAC).

KEYWORDS: Field experiment; Growth rate; Macrophyte; Model parameters; Risk assessment

INTRODUCTION

All life on earth, directly or indirectly, depends on primary producers, which play a key role in the structure and functioning of ecosystems (Wetzel, 2001). In freshwater bodies, aquatic macrophytes are considered a key driver for setting specific protection goals (European Food Safety Authority PPR Panel [EFSA Panel on Plant Protection Products and their Residues], 2013). Therefore, these organisms are required to be evaluated in the risk assessment of plant protection products (PPPs) and, in particular, of herbicides (European Commission [EC] Regulation [EU], 2013; European Food Safety Authority PPR Panel [EFSA Panel on Plant Protection Products and their Residues], 2013). Aquatic macrophytes are often classified by their growth forms (Maltby et al., 2010). *Lemna* sp. L. (Lemnaceae) is representative of nonsediment-rooted, free-floating, monocotyledonous macrophytes (Landolt, 1987). Its short generation time and good performance under laboratory conditions make this species well suited as a standard laboratory test species (International Organization for Standardization, 2005; Organisation for Economic Co-operation and Development [OECD], 2006). Therefore, it is part of the set of standard species in the Tier 1 risk assessment of herbicides, biocides, pharmaceuticals, and other chemicals whose mode of action targets aquatic primary producers (European Chemicals Agency [ECHA], 2018; European Commission [EC] Regulation [EU], 2013; US Environmental Protection Agency [USEPA], 2012). Where there is evidence

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in these cases that the test compound has herbicidal activity, a test with *Lemna* sp. must be performed according to the OECD Test Guideline 221 (*Lemna* sp. Growth Inhibition Test; OECD, 2006) or the USEPA Ecological Effect Test Guideline (Aquatic Plant Toxicity Test Using *Lemna* spp. OCSPP 850.4400). Such a standard test should provide information on the inhibition of growth rate and yield, based on frond numbers and on a second variable such as frond area, dry weight, or fresh weight under axenic conditions (EC, 2009; ECHA, 2018; European Commission [EC] Regulation [EU], 2013; European Food Safety Authority PPR Panel [EFSA Panel on Plant Protection Products and their Residues], 2013; OECD, 2006; USEPA, 2012).

A *Lemna* sp. growth inhibition test (International Organization for Standardization, 2005; OECD, 2006; USEPA, 2012) is an axenic test in which the growth of competing algae and bacteria is limited, while temperature, nutrient availability, and light conditions are managed to optimize growth rates over the test's seven-day duration. To refine the risk identified in standard tests, the regulatory community (European Food Safety Authority PPR Panel [EFSA Panel on Plant Protection Products and their Residues], 2013, 2018) considers toxicokinetic–toxicodynamic (TK-TD) and population models to be promising tools for analyzing and predicting the effects of a substance on *Lemna* sp. under more realistic conditions, for example, in situations of time-variable exposure. The TK-TD models can simulate laboratory tests for refined exposure analysis, which is an approach following the requirements of Tier 2C according to the European Food Safety Authority PPR Panel (EFSA Panel on Plant Protection Products and their Residues; 2013, 2018; see also Arts et al., 2021). They can be used in combination with population models to assess field scenarios and make predictions over longer time scales (European Food Safety Authority PPR Panel [EFSA Panel on Plant Protection Products and their Residues], 2018), as Schmitt et al. (2013) did for *Lemna* sp. The potential use of such models to address the variety of exposure profiles predicted, for instance, by the FOCUS Steps 3 and 4 models was shown at the MODELINK workshop (Hommen et al., 2016).

Several other *Lemna* sp. models (without a TK-TD component) have been developed in the past (Driever et al., 2005; Van Dyck et al., 2021; Van der Heide et al., 2006; Peeters et al., 2013; Scheffer et al., 2003). These ecological models were applied to study the effects of density dependence, competition with other primary producers, the effects of temperature, and stable states. Until now, these models could only be tested with limited datasets. Due to a lack of more comprehensive data, Schmitt et al. (2013) used datasets for the growth of *Lemna* sp. in ditches monitored over a growth period of two months in late spring (Driever et al., 2005). They combined data with air temperature and radiation data taken from the European meteorological database MARS and used N and P concentrations from ditches within the experiment's two-month time frame. However, model predictions will only be accepted in a regulatory context if they are properly evaluated and validated (Augusiak et al., 2014) and if it is demonstrated that the seasonal population dynamics observed in the field can be adequately simulated. *Lemna* data from existing mesocosm studies are also often limited to a few months during spring and summer. They are usually not designed to provide datasets to test macrophyte models. Therefore, the lack of TK-TD model testing with field data is an issue raised by several authors (European Food Safety Authority PPR Panel [EFSA Panel on Plant Protection Products and their Residues], 2018; Larras et al., 2022).

Given the absence of a multiseason time series for *Lemna* sp. growth under outdoor conditions, the aim of this project was to generate a dataset of the seasonal dynamics of *Lemna* sp. These include periods of growth during spring, abundance at the potential carrying capacity of aquatic ecosystems in spring and summer, due to density dependence when *Lemna* sp. fronds reach full coverage of the water surface, and potential declines in abundance and biomass in autumn and winter. A similar research question was raised for the sediment-rooted aquatic macrophyte *Myriophyllum spicatum* L. and was elaborated in another study using a different experimental system (Arts et al., 2021). The *Lemna* sp. dataset is intended to allow the testing and refinement of *Lemna* sp. population models for application in the risk assessment of PPPs and other chemical stressors, as well as in restoration programs to combat eutrophication and in the management of *Lemna* sp. where this species is invasive. Specific questions discussed in this article are:

1. How do growth rates of *Lemna* sp. under outdoor conditions compare with growth rates achieved in standard growth inhibition tests in the laboratory and how do they depend on environmental parameters?
2. What density limits are reached (maximum abundance)?
3. What are the dynamics of *Lemna* sp. over the seasons?
4. How can the datasets collected in this study be applied in a refined risk assessment framework?

MATERIALS AND METHODS

Field site and experimental setup

The study was conducted at the experimental field station "Sinderhoeve," Wageningen University and Research, The Netherlands (51°59′53″N, 5°45′12″E) from spring/early summer 2017 to spring/early summer 2019 (including two winters). Environmental conditions were realistic in terms of water quality (use of natural surface water) and weather (temperature and global radiation under outdoor conditions) and were representative of a warm, temperate climate, fully humid with warm summers (type Cfb in the Köppen–Geiger System, Kottek et al., 2006). The experiments did not involve any addition of toxicants, and the experimental systems were free from any historical contamination, because they had been cleaned after previous

experiments and were set up with fresh sediment (clay from an uncontaminated oxbow lake during the pre-experiments) and a fresh overlying water layer from the uncontaminated groundwater and rainwater basin at the Sinderhoeve field station.

The dynamics of biomass and numbers of fronds of *Lemna* sp. were studied using 100 small microcosms (Supporting Information File S1). Each microcosm was 45 cm in internal diameter, with a surface area of 0.174 m^2, a height of 67 cm, a water depth of 56.5 cm, and a volume of 26.56 L. The microcosms were buried in the ground to reduce fluctuations in water temperature and provide conditions comparable with a small (edge-of-field) pond or ditch. The *Lemna* microcosms were filled with water from the groundwater basin at the Sinderhoeve experimental station on 10 and 11 May 2017. This water is a mixture of groundwater and rainwater and is low in nutrient contents. Sediment (half–half mixture of natural clay and plant soil with slow-release nutrient bullets) was included only in the microcosms in the pre-experiment (see Supporting Information File S2 and next paragraph). The microcosms were seeded with *Lemna minor* fronds from a local population at the Sinderhoeve experimental station. It was also from these local populations that the *L. minor* plants were taken for the short-term growth experiments. During the study, the microcosms remained predominantly inhabited by *L. minor*. Because some low abundances of other *Lemna* species (e.g., the flat growth form of *Lemna gibba* L.) could not be excluded, we use *Lemna* sp. to address the taxonomic species in this article.

Nutrient levels

In 2017, a pre-experiment was performed in which several nutrient treatments were tested, with and without sediment. The aim was to find a balance between a sufficient nutrient supply to *Lemna* sp. fronds to facilitate good growth and prevent the growth of planktonic algae caused by excess nutrients being provided. These pre-experiments demonstrated that a sediment layer was not necessary and that nutrient levels could be optimized when only a water layer was provided (see Supporting Information File S2). Nutrient levels were adjusted in 2018 (Supporting Information File S2, Data report *Lemna* sp. Microcosm monitoring 2017) to achieve final target water concentrations of 0.5 mg P/L and 1.4 mg N/L. The phosphorus concentrations were three times higher than the concentrations we started with in 2017 (see Supporting Information File S2). The nitrogen-to-phosphorus (N:P) ratio (for moles) was set at 6, as it is important to have a relatively high N:P ratio in order not to favor blue-green algae, which cause extra nitrogen input by fixing nitrogen from the air. N and P were added to the microcosms weekly as NH_4NO_3 and K_2HPO_4 from stock solutions. Effects of nutrient levels on the growth of *Lemna* were additionally studied in the laboratory (Overvest, 2018). As a consequence, levels were adjusted slightly on 9 April 2018 to concentrations of 1.15 mg/L N and 0.192 mg/L P, at an N:P ratio of 6. The frequency of nutrient additions was intensified to twice a week.

Experimental approaches to generating the datasets

The population dynamics and short-term growth rates of *Lemna* sp. were studied using three different experimental setups (Table 1). All three started with 100 fronds of *Lemna* sp. per microcosm, which corresponds to 575 fronds m^{-2}. Plants were selected with 2–4 fronds each.

1. *Destructively sampled microcosms* were used to study the development of frond numbers, fresh weight, and dry weight of the 100 fronds of *Lemna* sp. introduced into different microcosms. In a first experiment, from July 2017 to March 2018, nutrient levels appeared to be too low to achieve a typical growth for "*Lemna* water bodies," so nutrients were added in November. This led to high abundance in December and allowed us to assess the effects of a frost period later in winter. A second study began in June 2018 and lasted one year. In this study, microcosms were harvested each week in summer, every two weeks in spring and autumn, and monthly in winter.
2. *Continuously monitored microcosms* included a set of five microcosms in which frond numbers were monitored for one year (weekly to monthly) for a total of 20 samplings. The aim of monitoring five microcosms continuously was to allow the calculation of interval growth rates for each individual microcosm, including density dependence. Therefore, these microcosms were not harvested for biomass assessment.
3. Finally, the third experimental setup included "*growth rate microcosms*," in which 100 fronds taken from the *Lemna* sp. stock population at the Sinderhoeve experimental station were introduced into microcosms that had just been harvested and were thus free of *Lemna* sp. These microcosms were harvested 1–2 weeks after inoculation. The aim of these short-term experiments was to study changes in potential growth rates of frond number and biomass over the different seasons without the limiting effect of density dependence.

Management of microcosms

To prevent disturbance of the *Lemna* sp. by birds and beetles, the microcosms were covered with a net in spring 2018 and spring 2019. From June 2018 onward, the microcosms were protected from beetle larvae by a fence and from birds by a scarecrow. The water level in the microcosms was maintained naturally, as above a level of +56.5 cm the water was discharged naturally into the surrounding grassland via a protected overflow. This protected overflow prevented the *Lemna* sp. fronds from being washed away. If needed, water was added weekly to maintain water level at +56.5 cm.

Setup of the microcosms

Before starting the experiments in July 2017 and June 2018, all 100 microcosms were cleaned and refilled with water from the groundwater basin and reseeded with 100 fronds of *L. minor* L. each from the same source

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TABLE 1 Population dynamics and growth rates of *Lemna* sp. measured in different experimental setups

Label	Approach	Focus	Periods (main datasets in bold)	Environmental factors monitored
Destructively Sampled Microcosms	Stocking 100 microcosms with 100 fronds each and harvesting different microcosms over time	Frond numbers and biomass in different microcosms over time	– 07.17–03.18 (prestudy to evaluate nutrient levels, data on winter decline during ice cover) – **06.18–05.19** (final dataset over the one year considered in this article)	Continuously: air temperature, solar radiation Up to weekly: water temperature, pH, conductivity, oxygen, turbidity Up to monthly: nutrients (in continuously monitored microcosms only)
Continuously Monitored Microcosms	Monitoring the same five microcosms over time	Frond numbers and growth rate of the same five microcosms over time	– **06.2018–05.2019**	
Growth Rate Microcosms	Set up with two or three microcosms with 100 fronds, harvested after 1–2 weeks	Frond numbers and biomass growth rates without density dependence	– 07.17–09.17 (low growth, not further considered) – **06.18–11.18** (final dataset over the one year considered in this article)	

population as that used in the previous experiment. Individual plants with 2–4 fronds were selected. With the restart of the three experiments in June 2018 (Table 1), five microcosms were also randomly selected for nondestructive sampling of frond numbers in order to collect a dataset using the same microcosms over time (continuously monitored microcosms). Due to an invasion of *Phyllopertha horticola*, a beetle whose larvae live in the grassland at the Sinderhoeve site, a second restart occurred on 6 June 2018, with additional measures to keep out the beetles (see the "Management of microcosms" section).

Lemna sp. endpoints, water parameters, and weather conditions

Monitoring parameters included frond numbers (FN), biomass (only in the destructively sampled microcosms: fresh weight [FW] and dry weight [DW]), water parameters (see below), percentage coverage of filamentous algae (floating algae bed [FLAB]), and weather data. Seasonal dynamics of short-term growth rates of fronds were calculated from the monitoring parameters (see the equation under "Evaluation").

Frond numbers were counted by hand. With increasing numbers of *Lemna* sp. fronds, representative subsamples were counted and extrapolated to the surface area of the *Lemna* cover. Subsamples were at least 5% of the total *Lemna*-covered surface area per microcosm. Wet and dry weights were always determined for the full *Lemna* sp. population in a sampled microcosm. After removing the *Lemna* sp. with a sieve from a sampled microcosm, wet weight of biomass was measured after blotting the samples with filter paper to remove excess water and placing them in preweighed aluminum foil or cups. Dry weight was measured by drying biomass samples at 60 °C (OECD, 2006) in an oven for at least 48 h.

The measurement of water parameters and weather conditions followed similar methods as applied by Arts et al. (2021). Electrical conductivity (EC), pH, dissolved oxygen (O_2), turbidity (Turb), temperature (T), and alkalinity of the water were measured at a depth of −10 cm in the water column of the sampled microcosms on each sampling date. The first three parameters (EC, pH, and O_2) were measured using a Multimeter HQ40-d (Hach) with electrode model CDC401 for measuring EC, electrode model 10103 for measuring pH, and electrode model LDO10103 for measuring oxygen (following Arts et al., 2021). Nephelometric turbidity was measured in nephelometric turbidity units (NTU) and temperature along with pH measurements (following Arts et al., 2021). Alkalinity was measured using a Compact Titrosampler 862. Nutrient concentrations were analyzed every few months but more regularly in the summers of 2017 and 2018, whereas light in the water column (as photosynthetically active radiation [PAR]) was measured occasionally to assess the overall light conditions in the microcosms (comparable with the method applied by Arts et al., 2021). The nutrient concentrations were analyzed using a segmented flow analyzer after extraction with H_2O/$CaCl_2$ or KCL and were used to determine if further nutrient additions were needed in the microcosms.

A weather station (HOBO RX3000 Station—CELL-3G) provided solar radiation data (Rad at 1.6 m height) in W/m^2 and air temperatures (air T at 1.5 m) in °C, which were used for

analyses in this study. Parameters were measured every 10 min but were used as daily solar radiation and daily mean air temperature.

Evaluation

The average specific growth rate is the change in the natural logarithm of a variable, for example, FN, FW or DW, divided by the time interval.

$$\mu_{i-j} = \ln(N_j) - \ln(N_i)/(j - i),$$

where μ_{i-j} is the average specific growth rate from time i to time j, and N_i, N_j is the measurement variable at time i or time j.

Growth rates were calculated only from the data obtained in the short-term growth experiments (growth rate microcosms) and in the continuously monitored microcosms, because only these experiments generated data from the same microcosms over time.

The *Lemna* sp. model (e.g., Schmitt et al., 2013) assumes fixed ratios between frond number and biomass as well as between FW and DW. The data from the destructively sampled microcosms were used to validate these assumptions and assess the variability in these ratios across the seasons.

Simple and multiple regression of growth rates

For the growth rates with (Experiment 2, continuously monitored microcosms) and without density dependence (Experiment 3, growth rate microcosms), simple linear regression analysis was conducted between the abiotic factors measured and *Lemna* sp. growth rates for FN in both datasets, and also for DW in the growth rate dataset. We regarded the growth rates as dependent variables, and the EC, pH, O_2, T, Turb, FLAB, Air T, Rad, N, P, and N:P ratios as independent variables. If measurements at the start and end of an interval were available, the means were used; in the other cases, we used the starting or end values. Regressions with N, P, and N:P ratios could be analyzed only for the continuously monitored microcosms because nutrient data were only sufficiently included in this dataset. Multiple regressions followed the approach as applied by Arts et al. (2021). They were conducted to determine whether a subset of the environmental factors could predict the observed FN growth rates. Microsoft Excel and SigmaStat for Windows v. 4.0 (Systat Software, Inc., 2016) were used for the statistical analysis (similar to the approach in Arts et al., 2021).

RESULTS

Environmental conditions

During the study, weather conditions were representative of a mild sea climate in temperate regions (see also Arts et al., 2021). Air temperature followed the seasonal variations in weather conditions and ranged from a minimum of −8 °C in winter to a maximum of 30 °C in summer. It comprised one frost period with ice cover (18 February–21 March 2018; Arts et al., 2021; see Supporting Information File S3). Water temperature followed a similar pattern, with fewer extremes, ranging from 4 °C in winter up to 22 °C in summer (Figure 1). Trends in water parameters in the destructively sampled microcosms and in the continuously monitored microcosms followed a similar pattern (see Figure 2 for the continuously monitored microcosm and Supporting Information File S4 for the destructively sampled microcosms). The pH of the water in

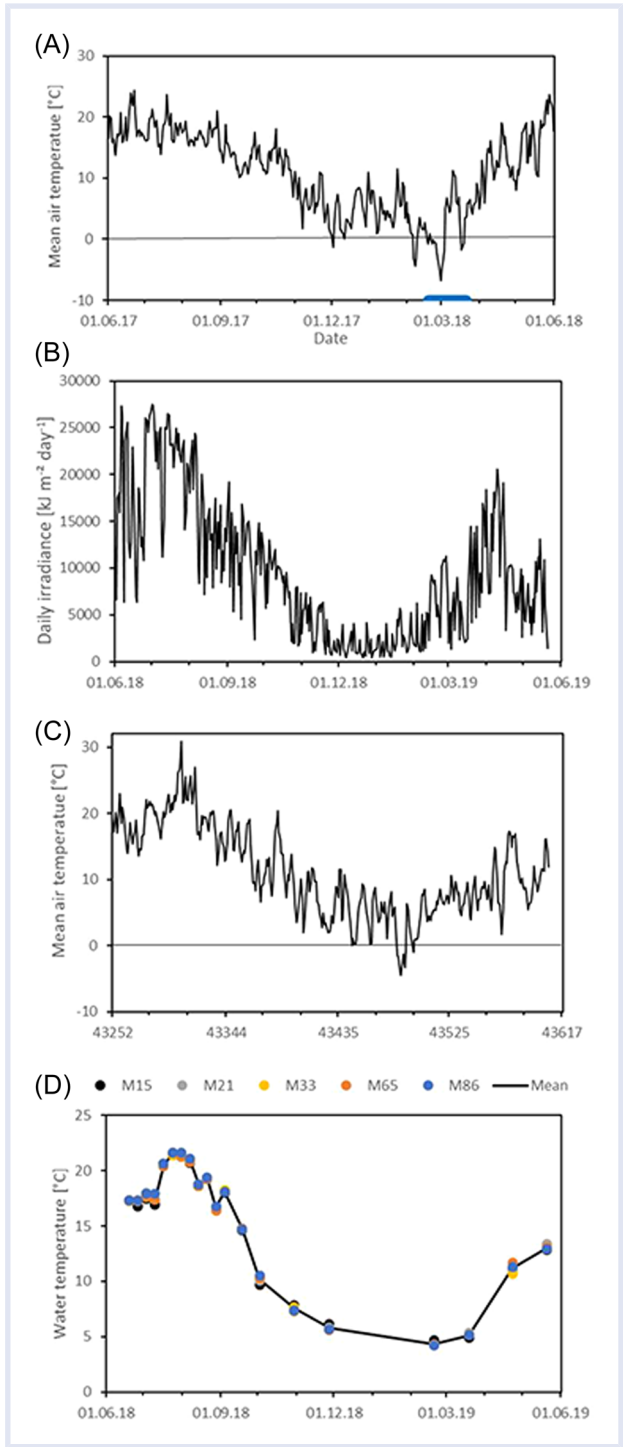

FIGURE 1 Light and temperature: (A) Mean air temperature in 2017/2018. The bold blue line along the *x*-axis indicates ice cover. (B) Daily irradiance 2018/2019. (C) Mean air temperature 2018/2019. (D) Water temperature measured in the five continuously monitored microcosms 2018/2019. Note that dates follow the British (European) notation, that is, day-month-year

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the microcosms decreased over time, from values greater than 10 in summer 2018 to values as low as 6 in spring 2019. However, not all microcosms exhibited this trend; in some of them, pH values remained between 9 and 10 (Figure 2). These latter microcosms did not develop a fully covering *Lemna* sp. mat or included floating algae mats (see Supporting Information File S4). O_2 (dissolved oxygen) levels decreased from 20 mg/L at the start of the experiments to almost 0 in some microcosms where *Lemna* (almost) reached 100% cover of the microcosm in June 2019. Conductivity varied between 130 and 200 µS/cm in 2018 but decreased in spring 2019 to between 50 and 150 µS/cm. Turbidity was variable over time and between microcosms and reached values up to 18 NTU in a single microcosm with low FN in spring 2019, although most values remained below 10 NTU. Photosynthetically active radiation in the water column varied from 40 to 900 µmol (see Supporting Information File S4). Nutrient concentrations in the water column varied between microcosms and over time, ranging from 0.01 to 1.1 mg/L for N-NH_4; 0.01 to 5.1 mg/L for N-NO_3; and 0.01 to 2.9 mg/L for P-PO_4. A rising trend in nutrient concentrations was observed until October 2018 (see Supporting Information File S4).

Seasonal dynamics of Lemna sp.

In the test starting in July 2017, *Lemna* sp. did not grow well until October, probably due to nutrient limitation. After nutrient levels (especially P) were raised in October, rates up to approximately 100 g DW m^{-2} were reached in December 2017 (Supporting Information Files S2 and S4). After a period of ice cover (18 February–21 March 2018), *Lemna* sp. plants became chlorotic and overgrown with fungi, and the populations collapsed.

In June 2018, the experiment was restarted with 100 fronds per microcosm, and increased nutrient levels were compared with 2017. Population growth of *Lemna* sp. was observed from June to the end of October (Figure 3A–C). On average, DW stabilized at a level of 100 g/m^2, and FN stabilized at a wide range of values in the continuously monitored and the destructively sampled microcosms, at between 10^3 and 10^5 fronds per microcosm. The absence of an ice cover in winter 2018/2019 probably allowed full coverage of *Lemna* sp. to be maintained during this winter, except in one of the continuously monitored microcosms (No. 65).

Ratios between biomass and frond numbers

The DW:FN ratios were between 0.1 and 1 mg DW:frond and exhibited a seasonal pattern (Figure 4), with the lowest values in summer (Figure 4). The highest value, 13 mg DW:frond, was measured in one sample in January 2018. The mean values for all samples were 0.56 and 0.21 mg DW:frond from samples taken from June to September.

The DW:FW ratio revealed a variable pattern (Figure 4) with the highest values (ca. 14%) measured in summer. The mean for all samples was 6.5%; in summer (June–September) the mean was 7%.

Growth rates

The *growth rate microcosms* were used to assess how *Lemna* sp. growth is affected mainly by temperature and light conditions without limiting nutrients or a limited surface area.

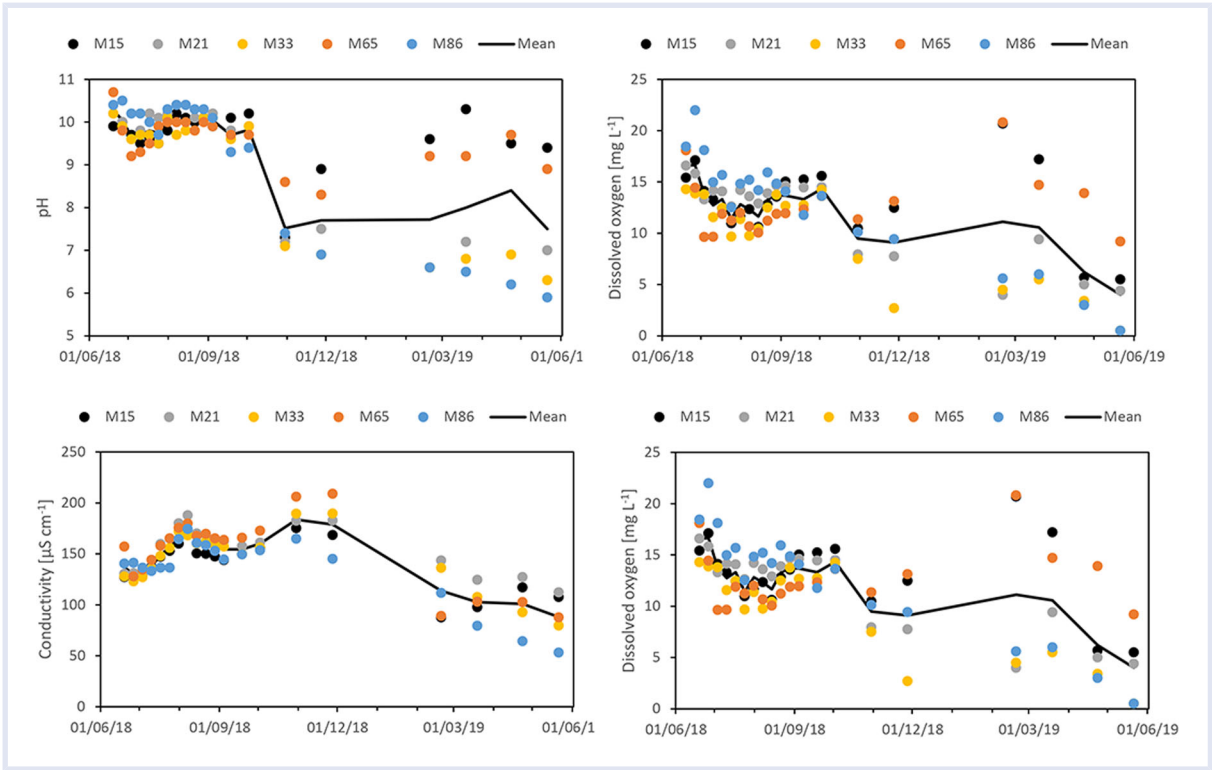

FIGURE 2 Water parameters in the continuously monitored microcosms. Note that dates follow the British (European) notation, that is, day-month-year

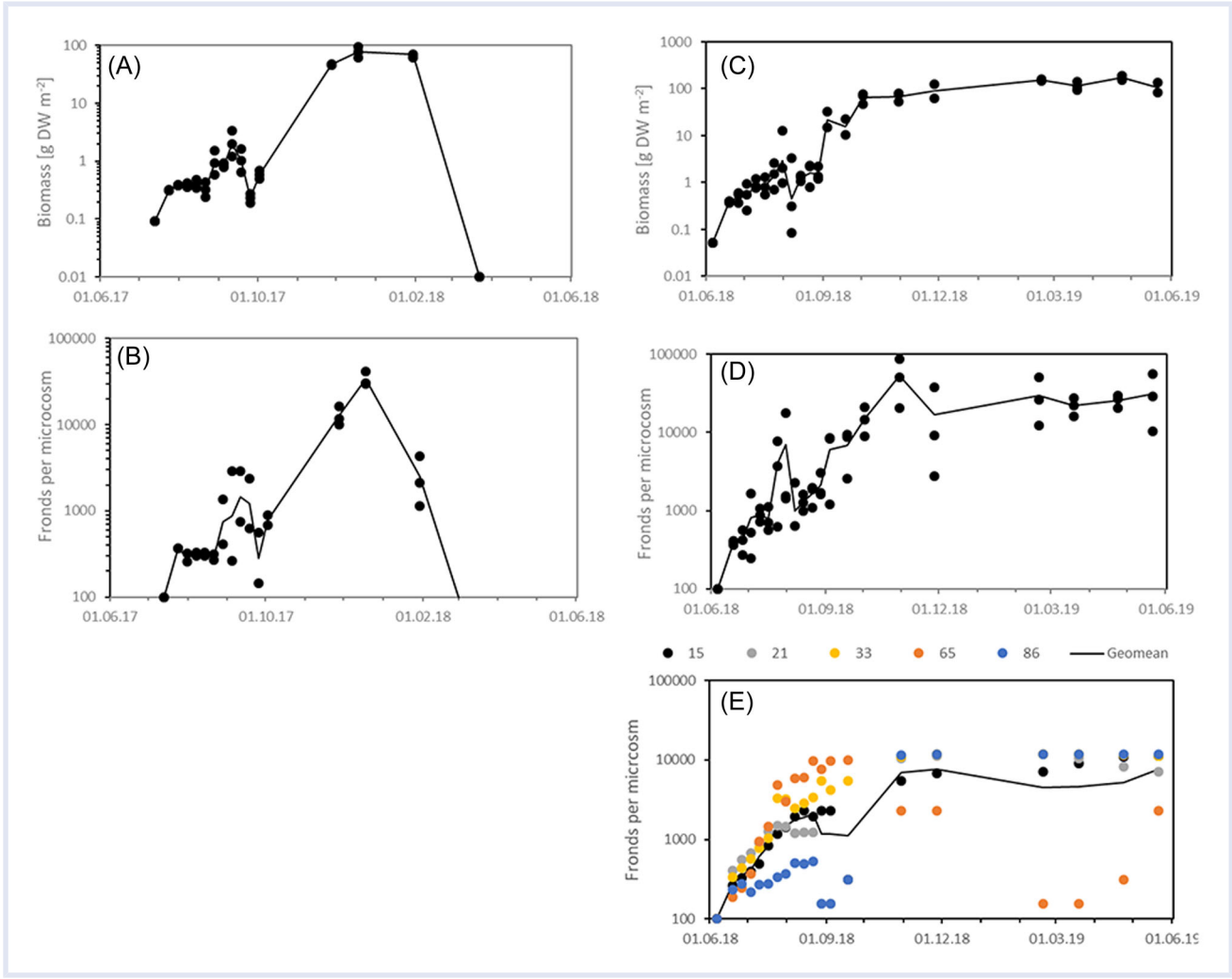

FIGURE 3 Biomass and frond numbers of *Lemna* sp. in the destructively sampled microcosms (A–D) and frond numbers in the continuously monitored microcosms (E), over time. Dots, individual microcosms; lines, geometric means. Note that dates follow the British (European) notation, that is, day-month-year

Therefore, empty microcosms were inoculated with 100 fronds, and the growth rate was determined after seven days until 4 September 2018 and after 14 or 28 days later in the year, when growth rates were lower. At the end of August 2018, the maximum growth rate of DW was 0.4 $days^{-1}$ and declined later to approximately 0.05 $days^{-1}$ at the end of November. The growth rates of FN were lower than those of DW, with a maximum of 0.22 $days^{-1}$. Linear regression revealed that the growth rate of FN was best explained by mean water temperature ($R^2 = 0.48$) and to a lesser extent by air temperature ($R^2 = 0.38$), pH ($R^2 = 0.28$), turbidity ($R^2 = 0.20$), or global radiation ($R^2 = 0.15$; Figure 5 and Supporting Information S4, sheet Regression of growth microcosms). Multiple linear regression with forward selection resulted in a model that included water temperature and pH with an R^2 of 0.76. The other factors did not significantly improve the prediction of the growth rate of FN. The coefficients of determination for the growth rate of DW were lower than for FN. The mean pH yielded the best linear regression of the DW growth rate ($R^2 = 0.33$), followed by water temperature ($R^2 = 0.28$), turbidity ($R^2 = 0.21$), air temperature ($R^2 = 0.16$), and irradiation ($R^2 = 0.06$). In multiple linear regressions with forward selection, the water temperature was the only factor added to the model ($R^2 = 0.50$). The discrepancy with the results of the simple linear models is caused by the different sample sizes ($n = 45$ for water temperature but $n = 33$ for the full dataset used for multiple regression).

In the *continuously monitored microcosms*, where nutrient limitation and density-dependence effects were also possible, the maximum growth rate for FN was approximately 0.17 $days^{-1}$, observed in two microcosms on 24 July 2018. However, the variability between microcosms and over time was large. In the same week, the growth rate in two other microcosms was approximately 0.03 $days^{-1}$, and negative growth rates were calculated the following week. Simple linear regression of this dataset revealed that the N:P ratio explained the largest part of the variance in the dataset ($R^2 = 0.19$, Figure 6), followed by nitrate-nitrite nitrogen concentration ($R^2 = 0.13$), global radiation ($R^2 = 0.07$), and air temperature ($R^2 = 0.03$). The R^2 values for radiation and temperature, which were lower than those found for the growth microcosms, may have been caused by the relatively stable abundance in four

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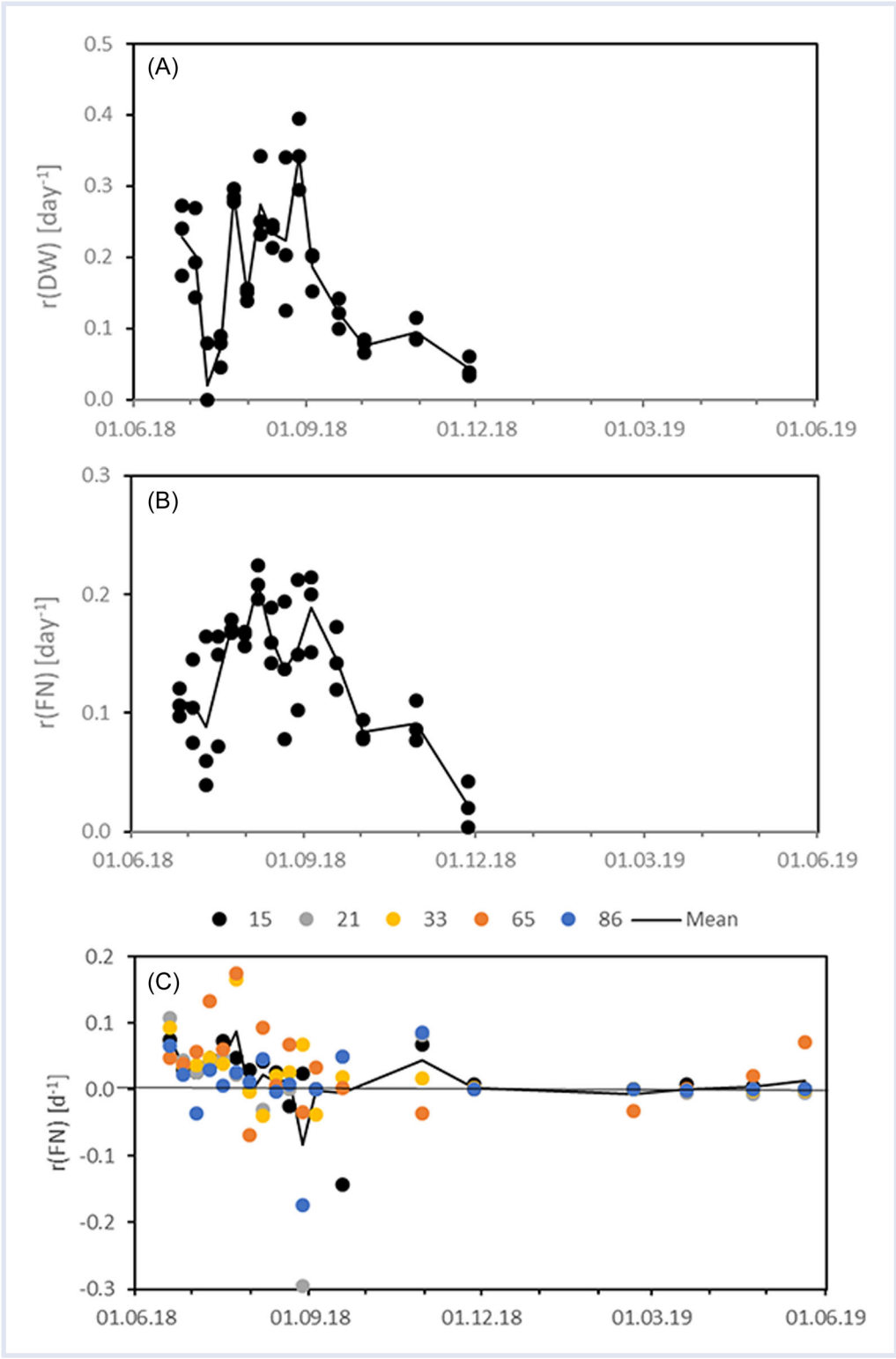

FIGURE 4 Growth rates of biomass (A) and frond number (B) in the growth rate microcosms and growth rates of frond number in the continuously monitored microcosms (C). Dots, individual microcosms; lines, geometric means. Note that dates follow the British (European) notation, that is, day-month-year

of the five microcosms from November 2018 to May 2015, despite the seasonal dynamics of these factors. Note that not all environmental parameters were measured at the same frequency. The regressions with the nutrient values were based on 34 data pairs from seven dates, whereas 89 to 100 data pairs were available for other parameters (see Supporting Information File S4). Multiple linear regression with forward selection including the nutrient variables ($n = 34$) revealed that the N:P ratio was the most important factor, whereas other factors did not significantly improve the model.

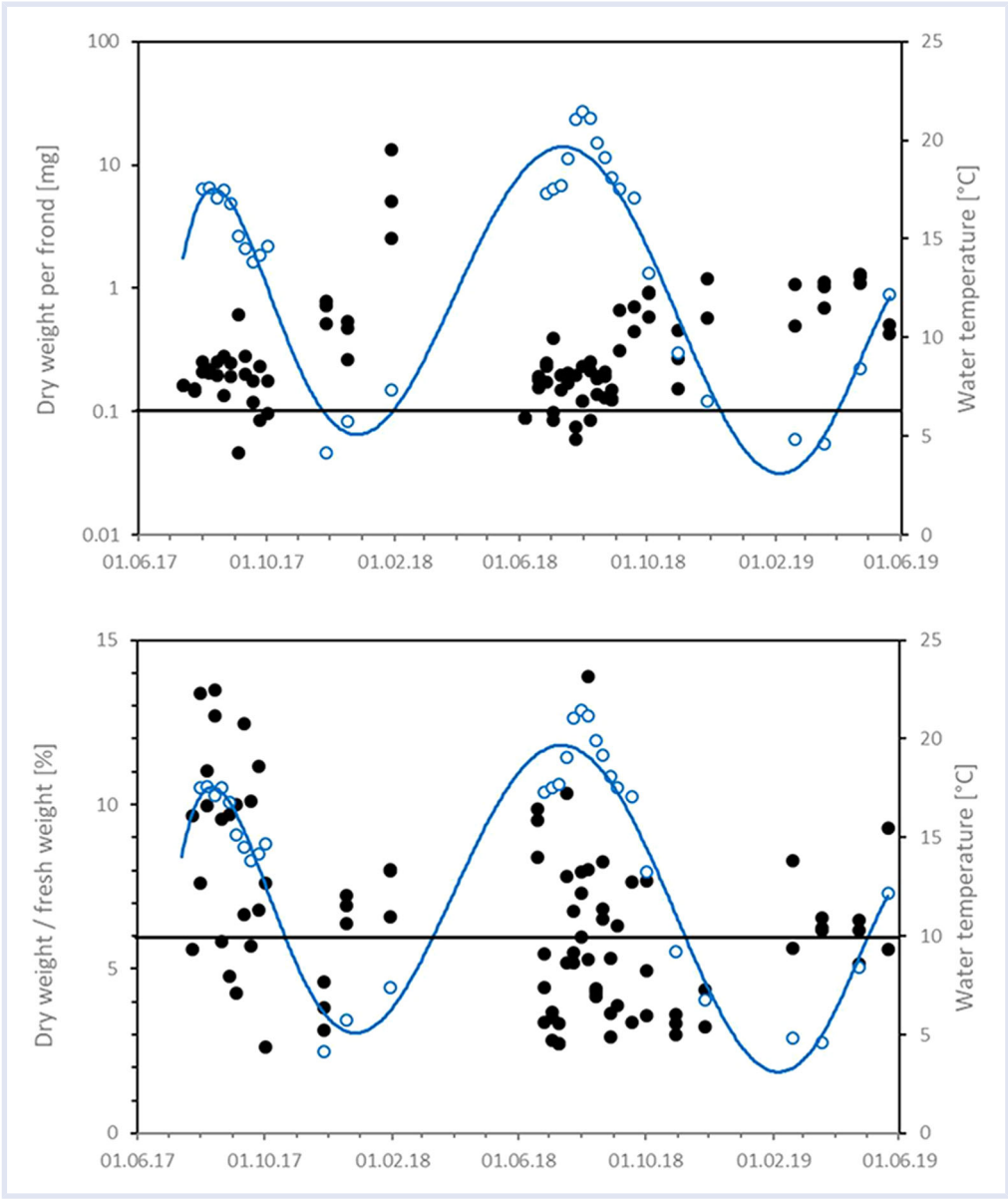

FIGURE 5 Dry-weight-to-frond number and dry-weight-to-fresh-weight ratios (black dots) in the harvested and/or destructively sampled microcosms, concerning all measurements. The horizontal lines represent the 0.1 mg DW:frond and 6% DW:FW ratios, respectively, assumed as fixed ratios in the model by Schmitt et al. (2013). The mean measured water temperature (open dots) and a polynomic fit are shown to indicate the seasons. Note that dates follow the British (European) notation, that is, day-month-year

When the nutrient variables were taken out of the dataset, and thus more data could be analyzed ($n = 89$), irradiance was the only factor selected. However, both regressions only explained a small part of the total variance (19% and 6%, respectively), and the prediction intervals were extremely broad (see Supporting Information File S4).

DISCUSSION

Lemna sp. growth under realistic outdoor conditions

The aim of these *Lemna* sp. experiments was to analyze population dynamics of *Lemna* sp. under realistic environmental conditions, expecting strong growth in spring, stable abundance due to density dependence in summer, and a decline in autumn and winter. We had to restart the experiments in 2018 due to a number of factors: (1) competition with other primary producers such as planktonic algae (in 2017) and FLAB (in 2018); and (2) disturbance by birds and an invasion of the beetle *Phyllopertha horticola*. In most microcosms, full coverage was reached in late summer/autumn in both years.

Lemna minor L. was introduced at the start of all experiments as 100 fronds and continued to be the dominant species in the microcosms over the experimental period. This is consistent with the parameters used by Schmitt et al. (2013) for the *Lemna* model. Because most literature information was found for *L. minor*, they decided to compile a consistent parameter set for this species. Therefore,

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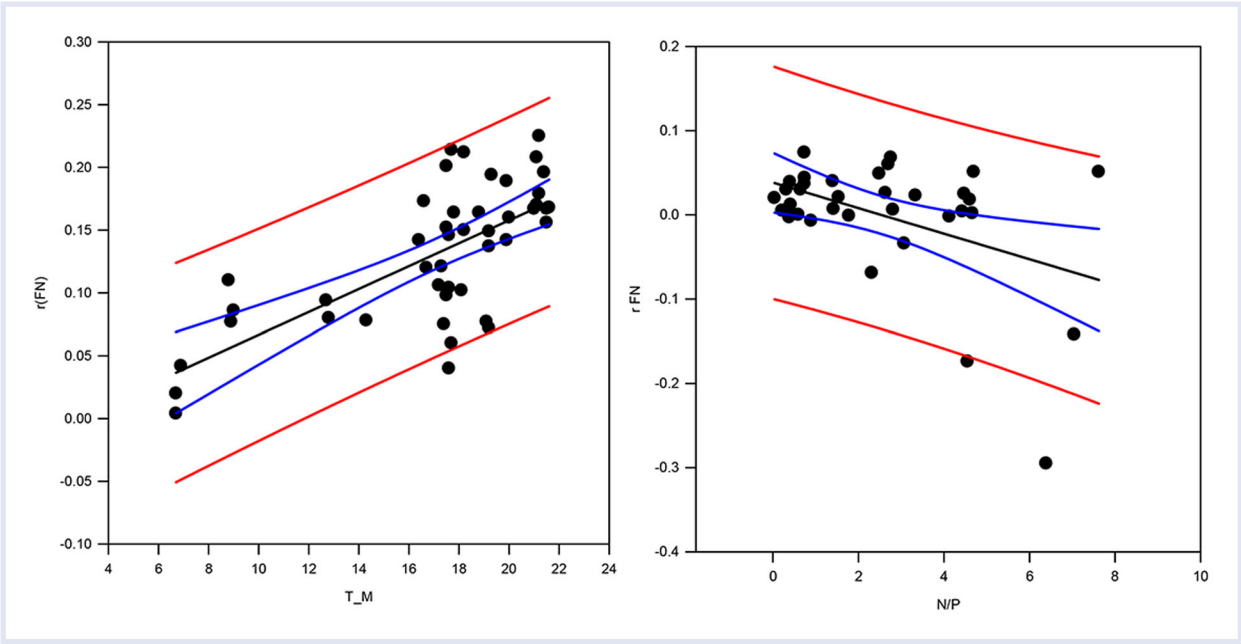

FIGURE 6 Simple linear regressions of frond number growth rates with the highest coefficient of determination. Left: growth rate (day^{-1}) as a function of the mean water temperature per interval (T-M [°C]) in the growth rate microcosms ($R^2 = 0.48$, $p < 0.001$). Right: growth rate (day^{-1}) as a function of the N:P ratio in the continuously monitored microcosms ($R^2 = 0.19$, $p = 0.008$). Inner blue curves: 95% confidence intervals; outer red curves: 95% prediction intervals

wherever possible, Schmitt et al. (2013) chose data for *L. minor*. In the remaining cases, data from an alternative *Lemna* species were used by Schmitt et al. (2013).

Finetuning the nutrient levels was considered a key factor in each of the three experimental setups. Because an excess of nutrients would have stimulated the growth of planktonic algae in situations where the *Lemna* sp. cover was incomplete, thereby increasing the competition with *Lemna* sp. fronds (Edwards et al., 1992; Szabó et al., 2005), the nutrient levels were balanced between offering sufficient nutrient supply to *Lemna* sp. fronds—to prevent nutrient limitation—and preventing the growth of planktonic algae. Algal blooms, caused by both filamentous algae and unicellular green algae, have been reported as the most important factors in limiting the growth of *Lemna* spp. through competition for nutrients and space (Edwards et al., 1992; Szabó et al., 2003). It is important to realize that the *Lemna* growth inhibition test in the laboratory (OECD, 2006) is an axenic test in which growth of algae is prevented. This is substantially different than the outdoor experiments we performed, which focused on good growth for *Lemna* without fully preventing the growth of other primary and competing producers (phytoplankton algae and FLAB). In their analyses, Roijackers et al. (2004) demonstrated that algae inhibited the growth of *Lemna* sp. by taking up N, P, and Fe, as well as by photosynthetically increasing the effect on pH. However, Roijackers et al. (2004) concluded that, in the long run, *Lemna* sp. will probably always expand sufficiently to outcompete the algae at high nutrient levels.

Environmental factors

In our study, growth rates of FN in the growth rate microcosms were best predicted by water temperature. Other studies have also concluded that temperature is one of the most important factors in determining the growth rates of free-floating macrophytes (Van Dyck et al., 2021; van der Heide et al., 2006; Landolt, 1987; Peeters et al., 2013). With increasing temperatures, the growth rate of *Lemna* increases approximately linearly to an optimum (Landolt, 1987). Van der Heide et al. (2006) presented a simple equation for describing the temperature-dependent growth of free-floating macrophytes, *L. minor* being one of them. The authors demonstrated that their simple three-parameter equation was highly predictive of the temperature-dependent growth of free-floating macrophytes. However, Van Dyck et al. (2021) optimized the growth model for *Lemna* sp. by using separate datasets for temperature, light intensity, photoperiod, and nutrients.

Lemna sp. depends on environmental factors but may also change them. It can deplete nutrients (Scheffer et al., 2003) and can obtain significant amounts of inorganic N through both roots and fronds (Cedergreen & Madsen, 2002), the so-called luxurious consumption. The plants acclimatize morphologically as well as physiologically to nitrogen availability in the surrounding water, with ammonium being preferred (Cedergreen & Madsen, 2003).

Lemna can also change the conductivity and pH of water (McLay, 1974), thus changing its own growth conditions (Landolt & Kandeler, 1987). This was confirmed by our observation that the pH of the water in the microcosms that were fully covered by *Lemna* sp. decreased over time, from values greater than 10 to values as low as 6. Thus, pH displayed a wide range of values (from 6 to 10) in the microcosms (Figure 2). When *Lemna* fully covers the water layer, CO_2 exchange between the water layer and the air is no longer possible, and *Lemna* sp. will exchange gases directly

with the air compartment. Over time, the natural trend of a *Lemna* population is toward a closed *Lemna* sp. mat. Such a closed mat forms a physical barrier to the water layer below, resulting in lower O_2 levels or even O_2 depletion, as well as less or no light penetration, lower pH, and lower electrical conductivity (Figure 2). The pH decrease is an indirect effect of reduced light availability in the water column, leading to less photosynthetically active algae. The duckweeds *L. minor* and *Spirodela polyrhiza* do not release any oxygen into the water on which they float (Filbin & Hough, 1985; Pokorny & Reijmánková, 1983). The mats limit photosynthesis in the water layer below them (Morris et al., 2004), resulting in extremely low oxygen concentrations. The prevailing anoxic conditions under a closed *Lemna* sp. mat suppress nitrification and the associated denitrification process, making ammonium the dominant nitrogen source (Boedeltje et al., 2005; Van Luijn et al., 1999).

In addition to the requirement of sufficient nutrients for its growth, *Lemna* sp. also requires specific ratios of nitrogen and phosphorus (Van Dyck et al., 2021). These authors found that the modified Hoagland solution (N:P ratio 7.73) sustained the growth of *L. minor* (measured either as FM or DM), whereas the growth rate did not change when the phosphorus concentration was reduced (N:P ratio 29.57). However, growth was more affected when the nitrogen concentration was lowered (N:P ratio 1.18; Van Dyck et al., 2021). In our study, we added nutrients to the microcosms at an N:P ratio of 6, which represents a favorable situation for growth, as supported by the findings of Van Dyck et al. (2021).

Lemna sp. field biomass and growth rates

Reported values for the biomass of *L. minor* recorded under field conditions range from 50 g/m^2 (Reimănkovă, 1975) to 537.6 g/m^2 of DW (Boedeltje et al., 2005). Schmitt et al. (2013) reported unpublished data from a ditch experiment at the same experimental facility where we performed our experiments, the Sinderhoeve. They found a value of 80 g/m^2 DW, which is in line with our findings of a biomass stabilized at a level around 100 g/m^2 in DW (Figure 3A and 3C) from October to May.

It is also known that frond size and surface:weight ratios in *Lemna* change when the plants are exposed to herbicides with various modes of action (Cedergreen & Streibig, 2005; Cedergreen et al., 2004). In the model presented by Schmitt et al. (2013), these ratios are assumed to be constant (0.1 mg DW:frond), as is the case under laboratory conditions. Many Lemnaceae species are able to form turions to survive unfavorable conditions. These fronds are smaller than normal fronds and contain more carbohydrates and fewer air spaces (Landolt, 1998). Our study revealed that the dry weight:frond ratio followed a seasonal pattern, with *Lemna* plants increasing in weight during autumn. This increase might result from the fronds containing more carbohydrates and fewer air spaces, as described by Landolt (1998), but it might also be a density-dependence effect: during growth periods it is more favorable for the plant to invest in new daughter fronds than in larger or heavier fronds.

Growth rates from laboratories have been used mainly as maximum growth rates in the published literature for analytical purposes and modeling development (Driever et al., 2005; Van der Heide et al., 2006; Njambuya et al., 2011; Scheffer et al., 2003; Van Dyck et al., 2021). Data on growth rates of *L. minor* under field conditions are extremely scarce. Peeters et al. (2013) used a maximum growth rate of 0.40 day^{-1} for biomass, based on data from Janse (1998) and Driever et al. (2005). Janse (1998) presented a range of growth rate values of 0.2–0.4 day^{-1}, based on data from an outdoor eutrophication experiment in experimental ditches at the Sinderhoeve (not our experiment). Thus, the growth data Janse (1998) used are real field data based on outdoor conditions. The range and maximum value presented by Janse (1998) are in accordance with our results for r(DW), which lie between 0.1 and 0.4 day^{-1} (Figure 4A).

Density-dependent growth

In our study, the destructively sampled microcosms reached a maximum DW of 191 g/m^2 in one of the microcosms on 23 April 2019. This is just above the maximum population density of 180 g/m^2 reported by Driever et al. (2005). They found a maximum relative growth rate of approximately 0.3 day^{-1} at a density of 9 g DW m^{-2}. This growth rate was measured under laboratory conditions. Under the outdoor conditions in our study, a maximum growth rate of 0.395 g DW day^{-1} was reached in the growth rate microcosms. Clatworthy and Harper (1962) studied three lemnids (*L. gibba*, *L. minor*, and *S. polyrhiza* [= *Lemna polyrhiza*]) as well as the floating fern *Salvinia natans*, under laboratory conditions in culture media. They observed that, in monocultures, the relative growth rates of all the species decreased as the density increased (self-crowding), and thus, the shortage of nutrients also increased. The mean frond weight also decreased in *L. minor* and *S. polyrhiza* but remained unaffected in *L. gibba*. Reddy and Debusk (1985) also observed a similar linear decline in the specific growth rate with increasing density of *L. minor* (from 0.118–0.273 day^{-1} at 30 g/m^2 to 0.01–0.05 day^{-1} at 130 g/m^2) and *S. polyrhiza* (from 0.08–0.237 day^{-1} at 20 g/m^2 to 0.002–0.01 day^{-1} at 100 g/m^2).

Regulatory importance of the long-term data series generated in this study

This study aimed to generate long-term data series that are ecologically relevant and can be used for testing and refining and/or as input parameters for *Lemna* sp. population models. In parallel with this study, another long-term data series has been generated for a macrophyte with a different growth form and therefore a different exposure to PPPs (Arts et al., 2021). The lack of long-term data series was identified as a problem for the application of population models in the risk assessment (Arts et al., 2021; European Food Safety Authority PPR Panel [EFSA Panel on Plant Protection Products and their Residues], 2018; Larras et al., 2022). Such models, also combined with TK-TD models, can fill a gap in the risk assessment if extrapolation of the effects of, for example, herbicides from laboratory

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tests to populations under field conditions over longer time frames is required and if effects of different experimental scenarios must be explored. Such options are part of the higher tier risk assessment framework of pesticides in which several refinement options are available (European Food Safety Authority PPR Panel [EFSA Panel on Plant Protection Products and their Residues], 2013). Although TK-TD models can supplement laboratory experiments and facilitate the prediction of the effects of diverse dynamic exposure patterns on *Lemna* sp. based on exponentially growing populations under standard laboratory test conditions (the so-called Tier 2C), population models for *Lemna* sp. are intended to predict the effects of a realistic exposure pattern on *Lemna* sp. populations under realistic environmental conditions (the so-called Tier 3–4 in the refined risk assessment framework).

For *Lemna* sp., a TK-TD model was published (Schmitt et al., 2013), which has been reviewed by the EFSA panel with the following conclusion: "If properly documented, the published *Lemna* model can be the basis for a compound-specific *Lemna* sp. model to evaluate the effects of field-exposure profiles in Tier-2C, particularly if in the Tier-1 assessment *Lemna* sp. is the only standard test species that triggers a potential risk" (European Food Safety Authority PPR Panel [EFSA Panel on Plant Protection Products and their Residues], 2018). Apart from extrapolating standard experimental results (based on chronic exposure) to other environmental scenarios, the original model by Schmitt et al. (2013) also includes the option to model *Lemna* sp. population dynamics under more realistic field conditions, that is, where growth is affected by light, temperature, nutrient concentrations, and density dependence. This provides the option to analyze the effects of the timing of expected exposure events over the seasons on the population dynamics of *Lemna* (a so-called Tier 3 approach). Seasonal variation is a feature of the climate in temperate regions. As this study has demonstrated, some *Lemna* sp. characteristics vary with the season, for example, DW per frond and DW:FW ratios. Therefore, it is of utmost importance to consider such seasonal variations in macrophyte growth and characteristics in this context. Long-term data series covering several seasons are necessary to calibrate or validate models as being fit for purpose for such extrapolations (see also Arts et al., 2021). Many herbicides enter aquatic systems during periods that are important for the sustainability of aquatic macrophyte populations: in spring, when temperatures and light conditions are becoming favorable for macrophyte growth, and in autumn, when biomass is decreasing and carbohydrates are building up in *Lemna* sp. plants, necessary to overcoming potential unfavorable conditions in winter (Landolt, 1998). In addition to the analysis of the effects of the timing of expected exposure events on *Lemna* sp. populations, a second potential application of a *Lemna* sp. population model is its integration as a module within an aquatic ecosystem model to consider indirect effects in a food web. A third and important potential regulatory application is to use it in Tier 4 to provide landscape-level risk estimations by coupling it to a spatially explicit exposure model, similar to the one performed to determine the effects of a pyrethroid on the survival of three sensitive aquatic arthropods (Buddendorf et al., 2023). The European Food Safety Authority Panel on Plant Protection Products and their Residues (European Food Safety Authority PPR Panel [EFSA panel on Plant Protection Products and their Residues], 2014) identifies field studies and landscape-level models as the highest tier (Tier 4) for the risk assessment, that is, with the highest level of realism providing results closest to the specific protection goals.

Although the TK-TD model for use at Tier 2C can be easily calibrated and validated using datasets from laboratory tests, the testing of a more complex population model is hampered by the availability of data on population dynamics in the field (European Food Safety Authority PPR Panel [EFSA Panel on Plant Protection Products and their Residues], 2018; Larras et al., 2022). Schmitt et al. (2013) used datasets covering only a relatively short period when *Lemna* sp. populations are in their exponential growth phase. The datasets here provide more detailed data on different seasons, including a relatively warm and an average winter, which can be used to further test and refine the model and thus increase confidence in its use in the environmental risk assessment of PPPs. The conditions under which the datasets in this article were collected are realistic for a wide geographical region with temperate climatic conditions (see also Arts et al., 2021). They can be used as model input, and the predicted dynamics can be compared with field observations. The data from the short-term experiments can be used to test the modules of models describing the dependence of growth on environmental factors, especially water temperature and the N:P ratio, which have been identified as important parameters for *Lemna* sp. growth. If datasets are needed for calibration and validation of models under Mediterranean or boreal conditions, similar datasets could be collected under these warmer and colder conditions.

CONCLUSION

- This study generated a time series of seasonal dynamics for the growth of *Lemna* sp. under environmental conditions in temperate regions.
- Growth rates in the field can reach values close to those required in the standard laboratory tests designed for high exponential growth, but are affected by light, temperature, nutrient availability, and density dependence. Variability between the populations in the individual microcosms was considerable, but water temperature was found to be a major driver in the short-term experiments without density dependence. The N:P ratio was the factor that best explained the growth rate in continuously monitored microcosms.
- In the microcosms, a maximum absolute abundance of approximately 190 g DW m^{-2} was reached, which is in line with values reported in the literature.

- Dynamics in the two monitored winters were different: After a few weeks of ice cover in the first winter, the population collapsed, whereas in the following winter, without ice cover, most populations maintained high abundance throughout.
- Dry-weight-to-frond ratios were highest in autumn and winter, probably as a result of the storage of reserves. In summer, the ratio was higher than that assumed by Schmitt et al. (2013) in most cases (average of 0.2 mg/frond compared with 0.1 mg/frond). Dry weight to fresh weight ratios varied between 3% and 14% and were thus similar to the value of 6% assumed by Schmitt et al. (2013).
- This study has generated ecologically relevant, long-term data series for testing and refining and/or as input parameters for *Lemna* sp. population models to be applied in the higher tiers of the risk assessment framework for PPPs.

AUTHOR CONTRIBUTION

Gertie H. P. Arts: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; writing—original draft; writing—review and editing. **Jasper van Smeden**: Investigation; methodology. **Marieke F. Wolters**: Investigation; methodology. **J. Dick M. Belgers**: Investigation; methodology. **Arrienne M. Matser**: Investigation; methodology. **Udo Hommen**: Conceptualization; data curation; formal analysis; methodology; visualization; writing—review and editing. **Eric Bruns**: Conceptualization; methodology; writing—review and editing. **Simon Heine**: Conceptualization; methodology; writing—review and editing. **Andreas Solga**: Conceptualization; methodology; writing—review and editing. **Seamus Taylor**: Conceptualization; methodology; writing—review and editing.

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CONFLICT OF INTEREST

The following authors certify that they have no conflicts of interest to declare: Gertie H. P. Arts, J. van Smeden, M. F. Wolters, J. D. M. Belgers, A. M. Matser, and U. Hommen. The following authors are members of CropLife Europe expert groups and are employed by commercial companies: E. Bruns, S. Heine, A. Solga, and S. Taylor.

DISCLAIMER

The peer review for this study was managed by the Editorial Board without the involvement of U. Hommen.

Open Data Badge

This article has earned an Open Data Badge for making publicly available the digitally shareable data necessary to reproduce the reported results. The data are available at https://doi.org/10.4121/d553a536-880a-4cd7-ae75-30c009ba00c9. Learn more about the Open Practices badges from the Center for Open Science: https://osf.io/tvyxz/wiki.

DATA AVAILABILITY STATEMENT

Photos of the microcosms, the results of the pre-experiments, the experimental datasets covering experimental data over two consecutive years including the regression results, and the weather data are available through an online repository: 4TU. Research data. https://doi.org/10.4121/d553a536-880a-4cd7-ae75-30c009ba00c9.

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SUPPORTING INFORMATION

Photos of the microcosms used to generate the *Lemna* growth data.

Results of the *Lemna* pre-experiments.

All weather data over the experimental period.

All measured data in the experiments.

REFERENCES

Arts, G. H. P., van Smeden, J., Wolters, M. F., Belgers, J. D. M., Matser, A. M., Hommen, U., Bruns, E., Heine, S., Solga, A., & Taylor, S. (2021). Seasonal dynamics of the macrophyte test species *Myriophyllum spicatum* over two years in experimental ditches for population modeling application in risk assessment. *Integrated Environmental Assessment and Management*, *18*(5), 1375–1386. https://doi.org/10.1002/ieam.4553

Augusiak, J., van den Brink, P. J., & Grimm, V. (2014). Merging validation and evaluation of ecological models to 'evaluation': a review of terminology and a practical approach. *Ecological Modelling*, *280*, 117–128.

Buddendorf, W. B., Wipfler, L., Beltman, W., Baveco, H., Braakhekke, M. C., Bub, S., Gergs, A., & Schad, T. (2023). Aquatic risks at the landscape scale: a case study for pyrethroid use in pome fruit orchards in Belgium. *Environmental Science & Technology*, *57*(41), 15608–15616.

Boedeltje, G., Smolders, A. J. P., Lamers, L. P. M., & Roelofs, J. G. M. (2005). Interactions between sediment propagule banks and sediment fluxes explain floating plant dominance in stagnant shallow waters. *Archiv für Hydrobiologie*, *162*(3), 349–362.

Cedergreen, N., & Madsen, T. V. (2002). Nitrogen uptake by the floating macrophyte *Lemna minor*. *New Phytologist*, *155*, 285–292.

Cedergreen, N., & Madsen, T. V. (2003). Nitrate reductase activity in roots and shoots of aquatic macrophytes. *Aquatic Botany*, *76*, 203–212.

Cedergreen, N., & Streibig, J. C. (2005). The toxicity of herbicides to non-target aquatic plants and algae: Assessment of predictive factors and hazard. *Pest Management Science*, *61*, 1152–1160.

Cedergreen, N., Spliid, N. H., & Streibig, J. C. (2004). Species-specific sensitivity of aquatic macrophytes towards two herbicides. *Ecotoxicology and Environmental Safety*, *58*, 314–323.

Clatworthy, J. N., & Harper, J. L. (1962). The comparative biology of closely related species living in the same area. V. Inter and intraspecific interference within cultures of *Lemna* sp. and *Salvinia natans*. *Journal of Experimental Botany*, *13*, 307–324.

Driever, S. M., van Nes, E. H., & Roijackers, R. M. M. (2005). Growth limitation of *Lemna minor* due to high plant density. *Aquatic Botany*, *81*, 245–251.

Edwards, P., Hassan, M. S., Chau, C. H., & Pacharaprakiti, C. (1992). Cultivation of duckweed in septage-loaded earthen ponds. *Bioresource Technology*, *40*, 109–117.

European Chemicals Agency (ECHA). (2018). *Guidance on the Biocidal Products Regulation Volume IV: Environment Part A: Information Requirements Version 1.2 May 2018*.

European Commission (EC). (2009). Regulation (EC) No. 1107/2009 of the European Parliament and the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. *Official Journal of the European Union*, *L 309*, 1–50.

European Commission (EC) Regulation (EU). (2013). No. 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with the Regulation (EC) No. 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. *Official Journal of the European Union*, *L 93*, 3.4.2013, 1–84.

European Food Safety Authority PPR Panel (EFSA Panel on Plant Protection Products and their Residues). (2013). Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. *EFSA Journal*, *11*(7), 3290. https://doi.org/10.2903/j.efsa.2013.3290

European Food Safety Authority PPR Panel (EFSA Panel on Plant Protection Products and their Residues). (2014). Scientific opinion on good modelling practice in the context of mechanistic effect models for risk assessment of plant protection products. *EFSA Journal*, *12*(3), 3589. https://doi.org/10.2903/j.efsa.2014.3589

European Food Safety Authority PPR Panel (EFSA Panel on Plant Protection Products and their Residues). (2018). Scientific Opinion on the state of the art of Toxicokinetic/Toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms. *EFSA Journal*, *16*(8), 5377. https://doi.org/10.2903/j.efsa.2018.5377

Filbin, G. J., & Hough, R. A. (1985). Photosynthesis, photorespiration, and productivity in *Lemna minor* L. *Limnology and Oceanography*, *30*(2), 322–334.

Hommen, U., Schmitt, W., Heine, S., Brock, T. C., Duquesne, S., Manson, P., Meregalli, G., Ochoa-Acuña, H., van Vliet, P., & Arts, G. (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*, 82–95.

International Organization for Standardization. (2005). ISO/DIS 20079, Water quality—Determination of the toxic effect of water constituents and waste water to duckweed (*Lemna minor*)—Duckweed growth inhibition test (ISO TC 147/SC 5/WG 5).

Janse, J. H. (1998). A model of ditch vegetation in relation to eutrophication. *Water Science and Technology*, *37*, 139.

Kottek, M., Grieser, J., Beck, C., Rudolf, B., & Rubel, F. (2006). World map of the Köppen–Geiger climate classification updated. *Meteorologische Zeitschrift*, *15*(3), 259–263. https://doi.org/10.1127/0941-2948/2006/0130

Landolt, E. (1987). *The family of Lemnaceae—A monographic study, Vol. I—Veröffentlichungen des Geobotanischen Instituts der ETH* (Vol. 2, p. 71). Stiftung Rübel.

Landolt, E. (1998). Lemnaceae. In K. Kubitzki (Ed.), *Flowering plants · monocotyledons. The families and genera of vascular plants* (Vol. 4). Springer. https://doi.org/10.1007/978-3-662-03531-3_28

Landolt, E., & Kandeler, R. (1987). *Biosystematic investigations in the family of Duckweeds (Lemnaceae), Vol. 4: the family of Lemnaceae—A monographic study, Vol. 2 (phytochemistry, physiology, application, bibliography). Veröffentlichungen des Geobotanischen Instituts der ETH* (p. 95). Stiftung Rübel.

Larras, F., Charles, S., Chaumot, A., Pelosi, C., Le Gall, M., Mamy, L., & Beaudouin, R. (2022). A critical review of effect modeling for ecological risk assessment of plant protection products. *Environmental Science and Pollution Research*, *29*, 43448–43500. https://doi.org/10.1007/s11356-022-19111-3

Maltby, L., Arnold, D., Arts, G., Davies, J., Heimbach, F., Pickl, C., & Poulsen, V. (2010). *Aquatic macrophyte risk assessment for pesticides. SETAC Europe Workshop AMRAP, Wageningen, Netherlands* (p. 135). SETAC Press & CRC Press, Taylor and Francis Group.

McLay, C. L. (1974). The distribution of duckweed *Lemna perpusilla* in a small Southern California lake: an experimental approach. *Ecology*, *55*(2), 262–276.

Morris, K., Harrison, K. A., Bailey, P. C. E., & Boon, P. L. (2004). Domain shifts in the aquatic vegetation of shallow urban lakes: The relative roles of low light and anoxia in the catastrophic loss of the submerged angiosperm *Valisneria americana*. *Marine and Freshwater Research*, *55*, 749–758.

Njambuya, J., Stiers, I., & Triest, L. (2011). Competition between *Lemna minuta* and *Lemna minor* at different nutrient concentrations. *Aquatic Botany*, *94*(4), 158–164. https://doi.org/10.1016/j.aquabot.2011.02.001

Organisation for Economic Co-operation and Development (OECD). (2006). *Lemna* sp. Growth Inhibition Test. OECD guidelines for the testing of chemicals (OECD/OCDE TG 221, p. 22). OECD.

Overvest, P. (2018). *The growth rate of Lemna. Preventing algae and optimizing Lemna* (p. 19). Bachelor of Science Thesis at Wageningen University (SGL-80812), Aquatic Ecology and Water Quality Management, AEW-2017-62.

Peeters, E. T. H. M., van Zuidam, J. P., van Zuidam, B. G., Van Nes, E. H., Kosten, S., Heuts, P. G. M., Roijackers, R. M. M., Netten, J. J. C., & Scheffer, M. (2013). Changing weather conditions and floating plants in temperate drainage ditches. *The Journal of Applied Ecology*, *50*, 585–593. https://doi.org/10.1111/1365-2664.12066

Pokorny, J., & Reijmánková, E. (1983). Oxygen regime in a fishpond with Duckweeds (*Lemnaceae*) and Ceratophyllum. *Aquatic Botany*, *17*, 125–137.

Reddy, K. R., & Debusk, W. F. (1985). Growth characteristics of aquatic macrophytes cultured in nutrient enriched water: II. *Azolla*, duckweed and *Salvinia*. *Economic Botany*, *39*(2), 200–205.

Reimănkově, E. (1975). Comparison of *Lemna gibba* and *Lemna minor* from the production ecological viewpoint. Short communication. *Aquatic Botany*, *I*, 423–427.

Roijackers, R., Szabo, S., & Scheffer, M. (2004). Experimental analysis of the competition between algae and duckweed. *Archiv für Hydrobiologie*, *160*(3), 401–412. https://doi.org/10.1127/0003-9136/2004/0160-0401

Scheffer, M., Szabo, S., Gragnani, A., van Nes, E. H., Rinaldi, S., Kautsky, N., Norberg, J., Roijackers, R. M. M., & Franken, R. J. M. (2003). Floating plant dominance as a stable state. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 4040–4045.

Schmitt, W., Bruns, E., Dollinger, M., & Sowig, P. (2013). Mechanistic TK-TD-model simulating the effect of growth inhibitors on *Lemna* populations. *Ecological Modelling*, *255*, 1–10.

Szabó, S., Roijackers, R., & Scheffer, M. (2003). A simple method for analysing the effects of algae on the growth of *Lemna* and preventing algal growth in duckweed bioassays. *Archiv für Hydrobiologie*, *157*, 567–575.

Szabó, S., Roijackers, R., Scheffer, M., & Borics, G. (2005). The strength of limiting factors for duckweed during algal competition. *Archiv für Hydrobiologie*, *164*, 127–140.

US Environmental Protection Agency (USEPA). (2012). *Ecological effects test guidelines OCSPP 850.4400: Aquatic plant toxicity test using Lemna spp.* (p. 24).

van der Heide, T., Roijackers, R. M. M., van Nes, E. H., & Peeters, E. T. H. M. (2006). A simple equation for describing the temperature dependent growth of free-floating macrophytes. Short communication. *Aquatic Botany*, *84*, 171–175.

Van Dyck, I., Vanhoudt, N., Batlle, J. V. I., Horemans, N., Nauts, R., Van Gompel, A., Claesen, J., & Vangronsveld, J. (2021). Effects of environmental parameters on *Lemna* minor growth: an integrated experimental and modelling approach. *Journal of Environmental Management*, *300*, 113705. https://doi.org/10.1016/j.jenvman.2021.113705

Van Luijn, F., Boers, P. C. M., Lijklema, L., & Sweerts, J. P. (1999). Nitrogen fluxes and processes in sandy and muddy sediments from a shallow eutrophic lake. *Water Research*, *33*(1), 33–42.

Wetzel, R. G. (2001). *Limnology: Lake and river ecosystems. Monograph* (3rd ed., p. 1006). Academic Press.

