

Growth Recovery of *Lemna gibba* and *Lemna minor* Following a 7-Day Exposure to the Herbicide Diuron

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Abstract In agricultural catchments, aquatic ecosystems can experience a pulse exposure to pesticides. Following such exposure, non-target organisms that are not extirpated may recover. This paper investigates the potential of two duckweed species (*Lemna minor* and *Lemna gibba*) to recover from a 7-day exposure to different concentrations (0.4–208 $\mu\text{g L}^{-1}$) of the herbicide diuron. There was significant inhibition in the growth and biomass after the initial 7-day exposure (e.g. frond number $\text{EC}_{50} = 59.2$ and $52.2 \mu\text{g L}^{-1}$ for *L. minor* and *L. gibba*, respectively). Following transfer to clean media, recovery (the highest concentration yielding no significant difference in the effect endpoint from the control) was observed for all effects endpoints at concentrations ranging 60–111 $\mu\text{g L}^{-1}$ for *L. minor* and 60–208 $\mu\text{g L}^{-1}$ for *L. gibba*. These results suggest that recovery is possible for primary producers at environmentally relevant concentrations considered significant in ecological risk assessment.

Keywords Catchment · Ecological risk assessment · Photosystem II · Pulse exposure · Resilience

Pesticides entering streams located in agricultural catchments typically occur as a pulse exposure to aquatic organisms (Reinert et al. 2002; Skark et al. 2004; Andrus

et al. 2013). However, toxicity data commonly used in ecological risk assessments (ERA) do not take such exposure profiles into account, often incorrectly assuming that an effect will remain once the exposure event has ceased. Essentially, there is limited consideration of either resilience of, or recovery by, an affected population of organisms (McCahon and Pascoe 1990; Posthuma et al. 2002; Reinert et al. 2002; Cedergreen et al. 2005). However, some regionally-focused ERA studies have incorporated these exposure profiles into their assessments (Solomon et al. 1996). Inclusion of recovery in ERAs provides more insight to the extent of temporal and spatial impacts that could be observed in these types of systems when exposed to specific pesticides. First, robust and useful data characterizing toxicity and subsequent recovery are needed. One herbicide warranting an examination of recovery for incorporation into ERAs is diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea).

Diuron is a herbicide attracting significant attention from global regulatory agencies (APVMA 2011) due to its potential for contamination of surface water in agricultural catchments. In Australia, diuron is commonly used in agriculture to protect crops, such as cotton, cereals, legumes, grass seed and sugar cane, from weeds (APVMA 2011). As a moderately-highly persistent herbicide in soil (half-life = 20–119 days), that is stable in water at pH 7–9 (EFSA 2005), it is one of the most efficacious inhibitors of photosystem II in target plants (Renger 1986; Zer and Ohad 1995; Krieger-Liszky and Rutherford 1998). As a consequence of its widespread use, diuron is commonly detected in agricultural streams in Australia and around the world. For example, studies in Europe (Lamoree et al. 2002; Dorigo et al. 2010), Asia (Balakrishnan et al. 2012) and North America (Field et al. 2003) have reported concentrations of diuron in surface waters in the range of

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0.03–28 $\mu\text{g L}^{-1}$; while studies in Australia have reported concentrations for the Gwydir River catchment of New South Wales [maximum concentrations of diuron = 0.1–82.1 $\mu\text{g L}^{-1}$ (Burns 2011)], and the Tully–Murray catchment of Queensland [maximum concentrations of diuron = 0.01–19.0 $\mu\text{g L}^{-1}$ (Bainbridge et al. 2009)]. The current recommended concentration limit to protect Australian aquatic ecosystems is 0.2 $\mu\text{g L}^{-1}$ (ANZECC 2000), which suggests there are concerns of impacts in these catchments. However, it is important to note that such studies have shown the exposure of diuron in these catchments is not chronic. Hydrograph analysis and spatial modeling work by Burns (2011) found that exposure events in the Gwydir River catchment are intermittent, being dependent on time of chemical use, rainfall events, soil, and agronomical management, with pulses lasting no longer than 6–7 days during periods of crop production (spring–winter; October–June).

The observed pulse exposure to diuron in agricultural catchments suggests that while effects may occur during the exposure phase, they may be reversible (i.e. recovery) once the exposure has ceased. Some ERAs have alluded to uncertainty in their risk estimates as a result of pulse exposure studies, indicating the reversibility of observed toxic effects caused by certain herbicides, especially for compounds that directly inhibit the photosystems, such as atrazine (Solomon et al. 1996) or diuron. No previous studies have been found that characterize recovery of duckweed from a catchment-specific exposure event simulated in the laboratory to diuron. However, this hypothesis has been explored extensively with atrazine by introducing variability in exposure duration and a recovery phase into testing with a variety of aquatic primary producers [green algae (Baxter et al. 2013, 2014), periphyton (Prosser et al. 2013), and duckweed (Brain et al. 2012)]. Haynes et al. (2000) reported some recovery of photosynthesis in a seagrass species after significant inhibition of photosynthesis following diuron exposure (0.1–80 $\mu\text{g L}^{-1}$) to diuron for 5 days. However, the ability of duckweed to recover following exposure to diuron, whereby growth measures in treated populations are no longer significantly different from the control populations, has not been reported.

To support the development of catchment-scale ERA, pulsed exposure of pesticides and subsequent potential recovery of impacted organisms in streams of agricultural catchments requires further characterization. Such information would allow limited management resources to be focused where recovery is most unlikely, as well as to identify those compounds for which rapid recovery is not likely. This paper reports on a laboratory study, using an optimized method of the 7-day exposure test for duckweed, to assess the degree of recovery by *Lemna minor* L. and

Lemna gibba L. placed in fresh media for 7 days following exposure to diuron. Study results will provide relevant ecotoxicity data for diuron to characterize the risk it poses in areas such as the Gwydir River catchment, as reported by Burns (2011), and in other regions where diuron is applied.

Materials and Methods

Laboratory cultures of *L. minor* and *L. gibba* were originally obtained from a laboratory colony culture at Aquatox (Guelph, ON, Canada). They were maintained separately under axenic conditions in 250-mL Pyrex Erlenmeyer flasks containing 100 mL sterile modified Hoagland's E+ growth media (pH 4.6, prepared in redistilled deionized water, using reagents of ACS grade or better; see ASTM (1991) for media preparation details). Cultures were kept under constant, uniform, cool fluorescent lights at room temperature ($23 \pm 2^\circ\text{C}$) and a selection of five healthy plants (exhibiting no chlorosis and irrespective of their number of fronds) were transferred aseptically to fresh sterile growth media contained in Erlenmeyer flasks every 7–10 days.

Diuron exposure media were prepared from a 1 mg L^{-1} diuron stock solution containing 1.25 g (± 0.1 mg) of 80 % a.i. w/w (DuPont, Canada) dissolved in 1 L of sterilized growth media. From this initial stock, exposure solutions of 0.3, 3, 25, 50, 100 and 200 $\mu\text{g L}^{-1}$ were prepared in growth media. Concentrations were chosen to reflect ecologically-relevant exposures, as well as those at which an effect would be expected. Concentrations of diuron in the stock solutions were confirmed in duplicate at the beginning of each test using an enzyme-linked immunosorbent assay (ELISA) test kit following manufacturer's instructions (Abraxis, USA) (Limit of detection of 0.03 $\mu\text{g L}^{-1}$).

Duckweed toxicity assays were conducted using a modified version of the semi-static (renewal) approach described by Greenberg et al. (1992). Replicate ($n = 6$) polystyrene Petri dishes (diameter: 100 mm \times height: 15 mm; Fisher Scientific, USA) containing 40 mL of exposure solution for each diuron concentration, plus controls, were prepared. Into each Petri dish, two plants with three fronds of either *L. minor* or *L. gibba* were transferred. As is typical in these types of studies (Brain et al. 2012), the effects endpoints measured were total number of plants and fronds, and fresh and dry weights. To represent the initial state (t_0), the total number of plants and fronds in each Petri dish, and the fresh and dry weights of separate sample populations were recorded. For the latter endpoints, a sample population, taken from the remaining stocks of *L. minor* and *L. gibba*, consisted of replicate ($n = 6$) samples of two plants with three fronds.

These plants were blotted dry on paper towels and weighed for fresh weight on an analytical balance (± 0.01 mg), then oven dried (60°C) in envelopes for 24 h prior to the determination of dry weight. Lids were placed on the Petri dishes and arranged in a randomized block design on trays lined with black cardboard (to eliminate the reflection of light from the white trays). Trays were then transferred to an E-15 Conviron growth chamber maintained at $25 \pm 2^\circ\text{C}$ and a light intensity of $111 \pm 12 \mu\text{E m}^{-2} \text{s}^{-1}$. After 4 days, exposure media was refreshed to (1) maintain the exposure concentration, (2) replenish nutrients and (3) counter the effect of evaporation.

At the end of 7 days, the total number of plants and fronds in each Petri dish ($n = 6$) were counted. All plants from three of the six replicates (randomly chosen) were removed from their dishes, gently blotted dry on paper towels, and weighed for fresh and dry weight. Plants in the remaining three replicates were used for recovery experiments.

From the remaining random three replicates, two visually viable three-frond plants were selected from each dish, rinsed gently in distilled water and placed into their new respective Petri dishes containing fresh Hoagland's (40 mL) growth media without diuron. Petri dishes were then returned to the same growth chamber for a 7-day recovery phase. Growth media was replaced after 4 days. After 7 days, the number of plants and fronds were counted, and the fresh and dry weights recorded.

The effect of diuron exposure on plant and frond numbers, wet and dry weights, and growth rates at the end of the tests were calculated after each 7-day test period. Specific rates of new growth of each species and the chosen endpoints for measuring effects were calculated following OECD (2006a, b) parametric and non-parametric statistical approaches were used to test differences between treatments. To compare treatments, assuming normality and equal variance, a parametric analysis of variance (ANOVA) followed by a Dunnett's test in SigmaPlot 11.0 (Systat Software Inc., USA) was performed. Where the assumption of normality and equal variance was not met, a Dunnett's non-parametric test was run. This allows for the determination of the lowest observed effect concentration (LOEC) ($p < 0.05$) and no observed effects concentration (NOEC) ($p > 0.05$) for each endpoint. The ANOVA-approach was also used for the recovery scenario, with the greatest treatment concentration of diuron where endpoints showed no significant difference from control ($p > 0.05$) referred to as the greatest observable recovery concentration (GORC).

Effects concentrations (EC_x) were estimated using a four parameter log-logistic regression (Eq. 1) with the *drc* package within R (Ritz and Streibig 2005; R Development Core Team 2011), where y refers to the percent inhibition,

c refers to the minimum inhibition response (%), d is the maximum inhibition response (%), x refers to the exposure concentration ($\mu\text{g L}^{-1}$), e is the EC_{50} or the median inhibition concentration ($\mu\text{g L}^{-1}$), and b refers to the slope of the curve at its midpoint.

$$y = c + \left(\frac{d - c}{1 + \exp\{b(\log(x) - \log(e))\}} \right) \quad (1)$$

Results

The mean percent difference between measured concentrations of diuron in test solutions and the nominal concentration was $18 \% \pm 12 \%$ (Table 1). Measured concentrations were used in all statistical analyses.

Mean *L. minor* and *L. gibba* plant and frond numbers, wet and dry weights, and growth rate, generally decreased with increasing concentration of diuron (Table 2). Control performance was considered adequate for both species according to the recommendations of Environment Canada (2006). The LOECs were found to occur in *L. minor* at $28 \mu\text{g L}^{-1}$ of diuron for the frond numbers and dry weights and $60 \mu\text{g L}^{-1}$ for the plant numbers and wet weight. For *L. gibba*, the LOEC for plant and frond numbers was 60 and $28 \mu\text{g L}^{-1}$ for wet and dry weight.

For *L. minor*, the ranking of calculated EC_x values varied by endpoint, with EC_{10} and EC_{25} values lowest for the wet and dry weights; while the EC_{50} s were most sensitive for dry weight and frond number (Table 3). Similarly for *L. gibba*, the ranking of calculated EC_x found EC_{10} values lowest for the dry weight and plant number; the dry weight had the lowest of the EC_{25} values; and the wet and dry weights were the most sensitive endpoints at the EC_{50} (Table 3). The corresponding range of EC_x calculated for the rates of new growth in each effect endpoint were found to be typically much greater than those value estimates for the raw numbers. In general, *L. gibba* was consistently more sensitive to diuron exposure than *L. minor*.

Significant recovery (i.e., a response that was not statistically different from control by ANOVA) was found to occur for treatment concentrations of diuron up to $60 \mu\text{g L}^{-1}$ for both species of duckweed (Table 4). A lack of full recovery (i.e., a response that was statistically difference from the control by ANOVA) was observed for the treatment concentrations of 111 and $208 \mu\text{g L}^{-1}$. Based on these results calculated GORCs for *L. minor* were $60 \mu\text{g L}^{-1}$ for the average frond number and $111 \mu\text{g L}^{-1}$ for plant number and wet and dry weights. For *L. gibba*, GORCs were $111 \mu\text{g L}^{-1}$ for the plant numbers, $208 \mu\text{g L}^{-1}$ for the frond numbers, and $60 \mu\text{g L}^{-1}$ for the wet and dry weight. Significant differences in rates of new growth were similar to the raw data, except for dry weight

Table 1 Nominal and measured ($n = 2$) concentrations ($\mu\text{g L}^{-1}$) of diuron in stocks of growth media

Nominal concentration ($\mu\text{g L}^{-1}$)	Measured concentration ($\mu\text{g L}^{-1}$)	Percent difference (%)
0	<LOD of 0.03 $\mu\text{g L}^{-1}$	Not applicable
0.3	0.4	34
3	2	-30
25	28	12
50	60	19
100	111	11
200	208	4

Table 2 Summary of *L. minor* and *L. gibba* plant and frond numbers and fresh and dry weights (mg)

Concentration of diuron ($\mu\text{g L}^{-1}$)	Plant number and rate of new growth (plant number day ⁻¹) (n = 6)	Frond number and rate of new growth (frond number day ⁻¹) (n = 6)	Wet weight (mg) and rate of new growth (mg day ⁻¹) (n = 3)	Dry weight (mg) and rate of new growth (mg day ⁻¹) (n = 3)
<i>L. minor</i>				
0	15 ± 3 (0.28 ± 0.03)	79 ± 6 (0.37 ± 0.01)	110.1 ± 15.4 (0.36 ± 0.01)	6.9 ± 1.6 (0.36 ± 0.01)
0.4	15 ± 3 (0.28 ± 0.04)	79 ± 11 (0.37 ± 0.02)	111.3 ± 24.7 (0.36 ± 0.01)	6.6 ± 0.9 (0.36 ± 0.01)
2	16 ± 3 (0.29 ± 0.03)	82 ± 11 (0.37 ± 0.02)	107.9 ± 21.1 (0.36 ± 0.02)	6.9 ± 0.5 (0.37 ± 0.02)
28	14 ± 3 (0.27 ± 0.04)	60 ± 6^a (0.33 ± 0.01^a)	81.4 ± 19.9 (0.32 ± 0.02)	4.2 ± 0.2^a (0.29 ± 0.02)
60	9 ± 1^a (0.22 ± 0.02^a)	44 ± 7 ^a (0.28 ± 0.02 ^a)	57.5 ± 9.8^a (0.27 ± 0.03)	2.8 ± 0.8 ^a (0.23 ± 0.03)
111	7 ± 2 ^a (0.17 ± 0.04 ^a)	25 ± 4 ^a (0.20 ± 0.03 ^a)	61.9 ± 15.4 ^a (0.28 ± 0.06)	1.7 ± 0.5 ^a (0.16 ± 0.06)
208	4 ± 1 ^a (0.08 ± 0.06 ^a)	11 ± 2 ^a (0.09 ± 0.03 ^a)	8.6 ± 4.7 ^a (0.00 ± 0.15^a)	0.2 ± 0.2 ^a (0.00 ± 0.15^b)
<i>L. gibba</i>				
0	15 ± 3 (0.28 ± 0.03)	74 ± 12 (0.36 ± 0.02)	178.8 ± 14.0 (0.35 ± 0.01)	9.2 ± 0.9 (0.35 ± 0.01)
0.4	13 ± 4 (0.27 ± 0.04)	67 ± 19 (0.34 ± 0.04)	172.1 ± 7.6 (0.35 ± 0.02)	9.1 ± 0.5 (0.35 ± 0.02)
2	14 ± 3 (0.28 ± 0.03)	67 ± 13 (0.34 ± 0.03)	169.9 ± 37.5 (0.35 ± 0.01)	8.6 ± 0.7 (0.34 ± 0.01)
28	10 ± 4 (0.23 ± 0.05)	53 ± 9 (0.31 ± 0.02)	130.1 ± 9.7^a (0.31 ± 0.02)	5.6 ± 0.2^a (0.28 ± 0.02^a)
60	9 ± 2^a (0.21 ± 0.03^a)	39 ± 9^b (0.26 ± 0.03^b)	86.1 ± 22.1 ^a (0.25 ± 0.02^a)	3.6 ± 0.7 ^a (0.21 ± 0.02 ^a)
111	7 ± 2 ^a (0.16 ± 0.04 ^a)	24 ± 3 ^b (0.20 ± 0.02 ^b)	67.9 ± 18.3 ^a (0.21 ± 0.01 ^a)	1.8 ± 0.1 ^a (0.12 ± 0.01 ^a)
208	5 ± 1 ^a (0.11 ± 0.02 ^a)	14 ± 1 ^b (0.12 ± 0.01 ^b)	15.8 ± 2.7 ^a (0.01 ± 0.02 ^a)	0.9 ± 0.1 ^a (0.01 ± 0.02 ^a)

Mean ± SD at the end of 7-day exposure to different concentrations of diuron ($\mu\text{g L}^{-1}$), rates of new growth (presented in brackets, in units of measurement units per day), and the NOEC (first number in bold) and LOEC (second number in bold), respectively designated as those concentrations of diuron where the effects were not significantly different ($p < 0.05$) and the lowest treatment concentration significantly different ($p > 0.05$) from the control

^a Significant differences ($p < 0.05$) from the control based on Dunnett's ANOVA test, assuming normality and equal variance ($p > 0.05$)

^b Significant differences based on Dunnett's ANOVA rank test, not assuming normality and equal variance ($p < 0.05$)

of *L. minor* and fresh and dry weight of *L. gibba* where differences were observed for concentrations of 60, 208 and 111 $\mu\text{g L}^{-1}$, respectively.

For *L. minor*, the maximum inhibition relative to the control at 208 $\mu\text{g L}^{-1}$ for plant and frond counts and wet and dry weights were 68 %, 61 %, 46 % and 71 %, respectively (Table 4). For *L. gibba*, the maximum inhibition relative to control at 208 $\mu\text{g L}^{-1}$ for plant and frond counts and wet and dry weights were 39 %, 14 %, 43 %, and 38 %, respectively. This implies that *L. gibba* appears to recover from inhibition due to diuron to a greater extent than *L. minor* within 7 days.

Discussion

Exposure to diuron resulted in inhibition of growth in plant and frond numbers for populations of *L. minor* and *L. gibba* that also translated to reduced wet and dry weights as treatment concentration increased. Our results (LOECs and EC₅₀s) are similar to those reported by others, i.e., within twofold. Teisseire et al. (1999) reported a reduction in *L. minor* dry weight yield (7-day LOEC = 5 $\mu\text{g L}^{-1}$ and estimated EC₅₀ = 25 - $\mu\text{g L}^{-1}$) and Okamura et al. (2003) reported reduction in frond number for *L. minor* and *L. gibba* (7-day EC₅₀ = 29 and 30 $\mu\text{g L}^{-1}$, respectively) following exposure to diuron.

Table 3 Summary of *L. minor* and *L. gibba* EC₁₀, EC₂₅ and EC₅₀ for plant and frond number, and wet and dry weight estimated from a four-parameter logistic model with the parameters (from Eq. 1)

Endpoint	EC ₁₀ (µg L ⁻¹)	EC ₂₅ (µg L ⁻¹)	EC ₅₀ (µg L ⁻¹)	Model parameters
<i>L. minor</i>				
Plant number	27.3 (8.7)	48.4 (9.6)	85.8 (12.1)	b = -1.918; c = 0; d = 100; EC ₅₀ = 85.8
Growth rate	40.7 (8.7)	74.5 (9.2)	136.3 (12.3)	b = -1.819; c = 0; d = 100; EC ₅₀ = 136.3
Frond number	15.8 (2.6)	30.6 (3.2)	59.2 (3.7)	b = -1.667; c = 0; d = 100; EC ₅₀ = 59.2
Growth rate	34.6 (3.5)	63.7 (3.8)	117.4 (4.5)	b = -1.799; c = 0; d = 100; EC ₅₀ = 117.4
Wet weight	12.8 (6.4)	28.9 (9.0)	65.3 (11.8)	b = -1.350; c = 0; d = 100; EC ₅₀ = 65.3
Growth rate	103.4 (31.6)	111.8 (5.0)	120.8 (43.2)	b = -14.17; c = 0; d = 100; EC ₅₀ = 120.7
Dry weight	9.7 (3.7)	19.2 (4.7)	37.9 (5.6)	b = -1.613; c = 0; d = 100; EC ₅₀ = 37.9
Growth rate	36.9 (17.0)	53.1 (17.5)	76.6 (17.3)	b = -3.005; c = 0; d = 100; EC ₅₀ = 76.6
<i>L. gibba</i>				
Plant number	8.1 (4.7)	22.1 (7.6)	60.8 (11.0)	b = -1.089; c = 0; d = 100; EC ₅₀ = 60.8
Growth rate	16.5 (5.4)	48.8 (8.1)	144.0 (17.5)	b = -1.015; c = 0; d = 100; EC ₅₀ = 144.0
Frond number	9.6 (2.9)	22.4 (4.1)	52.2 (4.7)	b = -1.298; c = 0; d = 100; EC ₅₀ = 52.2
Growth rate	24.2 (2.9)	55.1 (3.6)	125.2 (5.4)	b = -1.338; c = 0; d = 100; EC ₅₀ = 125.2
Wet weight	12.0 (3.9)	24.9 (5.1)	51.8 (6.2)	b = -1.503; c = 0; d = 100; EC ₅₀ = 51.8
Growth rate	38.3 (10.1)	62.4 (10.5)	101.8 (10.0)	b = -2.248; c = 0; d = 100; EC ₅₀ = 101.8
Dry weight	9.0 (2.3)	17.8 (2.9)	35.1 (3.2)	b = -1.610; c = 0; d = 100; EC ₅₀ = 35.1
Growth rate	22.3 (3.0)	40.0 (3.4)	71.5 (3.8)	b = -1.889; c = 0; d = 0; EC ₅₀ = 71.5

Standard error in brackets

Relative to the 7-day exposure scenario, recovery in plant and frond numbers and wet and dry weight in *L. minor* and *L. gibba* were observed at the NOEC, LOEC, EC₁₀, EC₂₅ and EC₅₀ concentration thresholds (Table 4) typically deemed ecologically detrimental in ERAs at the end of the 7-day recovery period. In this study, recovery was observed for all endpoints at concentrations up to 60 µg L⁻¹ of diuron but that level of recovery was not observed for some endpoints at 111 and 208 µg L⁻¹ diuron. These data support the idea that toxicity of diuron to *L. minor* and *L. gibba*, via inhibition of photosynthesis activity, is not permanent at levels similar to the toxicity endpoints used in ERAs. This onset of recovery is likely to be the result of reversibility in the exposure effects, facilitated by the introduction of the affected macrophytes to the clean growth media (Izawa and Good 1965; van Rensen 1982), except where the toxic effect was sufficient to overwhelm the ability to recover within the time assessed (≤7 days). Such a response has been reported in pulse studies involving *L. minor* and *L. gibba* exposed to other PSII herbicides. Brain et al. (2012) reported immediate recovery of growth rates of *L. gibba* exposed to atrazine [5–160 µg L⁻¹ for different lengths of time (1–14 days)]. Furthermore, they also reported no onset of obvious phytocidal effects (e.g. chlorosis or necrosis) during *L. gibba* exposure to atrazine. Effects from atrazine on *L. gibba*

were concluded to be phytostatic in nature and reversible, with time to recovery being independent of exposure duration, with the exception of the highest concentrations. Further to these observations, Cedergreen et al. (2005) suggested that oxidative damage to the PSII in *L. minor* caused by the PSII herbicide terbutylazine could be repaired resulting in restoration of photosynthetic activity within 24 h. Importantly, the restoration of PSII activity in these studies rapidly translated into organism growth, which is consistent with results presented in this study. These rapid changes in growth suggest the effect of diuron on photosynthesis is not permanent. This is likely due to rapid elimination of diuron from the affected plant cells due to the non-covalent nature of its binding to the Q_B translating into reversibility in the mode of action when plants were introduced to clean growth media (van Rensen 1982; Vallotton et al. 2008).

The ability of *L. minor* and *L. gibba* to recover from a 7-day pulse of diuron at treatment concentrations deemed significant (e.g. NOEC, LOEC, EC₁₀, EC₂₅ and EC₅₀), has implications for catchment-based ERAs. As methods for undertaking catchment-based ERAs mature, characterizing uncertainties in both exposure and effects analyses, such as the potential for recovery, requires a more locally explicit understanding of environmental dynamics. In a large-scale ERA, Solomon et al. (1996) considered the uncertainty

Table 4 Summary of mean \pm SD of *L. minor* and *L. gibba* plant and frond numbers, and fresh and dry weights (mg), together with rates of new growth (presented in brackets in units of measurement units per day) following 7 days of recovery from exposure to different concentrations of diuron ($\mu\text{g L}^{-1}$) in Hoagland's growth media

Exposure concentration of diuron ($\mu\text{g L}^{-1}$)	Plant number and rate of new growth (plant number day^{-1}) (n = 3)	Frond number and rate of new growth (frond number day^{-1}) (n = 3)	Wet weight (mg) and rate of new growth (mg day^{-1}) (n = 3)	Dry weight (mg) and rate of new growth (mg day^{-1}) (n = 3)
<i>L. minor</i>				
0	23 \pm 6 (0.35 \pm 0.04)	132 \pm 18 (0.44 \pm 0.02)	135.5 \pm 20.1 (0.44 \pm 0.04)	15.1 \pm 3.8 (0.47 \pm 0.04)
0.4	23 \pm 7 (0.35 \pm 0.04)	148 \pm 9 (0.46 \pm 0.01)	163.4 \pm 12.3 (0.46 \pm 0.00)	14.7 \pm 1.0 (0.47 \pm 0.00)
2	21 \pm 3 (0.34 \pm 0.02)	137 \pm 13 (0.45 \pm 0.01)	154.4 \pm 14.6 (0.46 \pm 0.01)	13.1 \pm 2.0 (0.45 \pm 0.01)
28	16 \pm 1 (0.30 \pm 0.01)	112 \pm 11 (0.42 \pm 0.01)	127.3 \pm 17.5 (0.43 \pm 0.01)	10.5 \pm 1.5 (0.42 \pm 0.01)
60	18 \pm 6 (0.31 \pm 0.04)	118 \pm 11 (0.43 \pm 0.01)	127.3 \pm 8.8 (0.43 \pm 0.02)	12.0 \pm 1.1 (0.44 \pm 0.02)
111	15 \pm 2 (0.29 \pm 0.02)	93 \pm 12 ^a (0.39 \pm 0.02 ^a)	109.9 \pm 21.8 (0.41 \pm 0.03)	8.7 \pm 2.0 (0.39 \pm 0.03^a)
208	8 \pm 1 ^a (0.20 \pm 0.01 ^a)	55 \pm 9 ^a (0.32 \pm 0.02 ^a)	75.4 \pm 19.2 ^a (0.35 \pm 0.03 ^a)	4.9 \pm 1.2 ^b (0.31 \pm 0.03 ^a)
<i>L. gibba</i>				
0	16 \pm 3 (0.29 \pm 0.02)	91 \pm 14 (0.39 \pm 0.02)	278.7 \pm 39.5 (0.39 \pm 0.02)	16.1 \pm 3.2 (0.39 \pm 0.02)
0.4	15 \pm 2 (0.29 \pm 0.02)	95 \pm 21 (0.39 \pm 0.03)	267.6 \pm 38.6 (0.38 \pm 0.02)	16.2 \pm 1.7 (0.39 \pm 0.02)
2	15 \pm 1 (0.29 \pm 0.01)	95 \pm 10 (0.39 \pm 0.02)	283.0 \pm 51.0 (0.39 \pm 0.02)	15.9 \pm 1.6 (0.39 \pm 0.02)
28	15 \pm 2 (0.29 \pm 0.02)	92 \pm 5 (0.39 \pm 0.01)	288.3 \pm 21.6 (0.39 \pm 0.01)	18.0 \pm 0.8 (0.41 \pm 0.01)
60	14 \pm 2 (0.27 \pm 0.02)	87 \pm 5 (0.38 \pm 0.01)	227.9 \pm 128 (0.36 \pm 0.01)	14.2 \pm 0.5 (0.37 \pm 0.01)
111	17 \pm 3 (0.30 \pm 0.02)	93 \pm 2 (0.39 \pm 0.00)	181.1 \pm 23.5 ^a (0.33 \pm 0.02)	12.2 \pm 0.9 ^a (0.35 \pm 0.02)
208	10 \pm 1 ^a (0.23 \pm 0.01 ^a)	77 \pm 5 (0.37 \pm 0.01)	162.6 \pm 26.5 ^a (0.31 \pm 0.02)	10.1 \pm 1.3 ^a (0.32 \pm 0.02 ^a)

The greatest observable recovery concentration (GORC) is designated in bold as the highest treatment concentrations not significantly different from the control ($p < 0.05$)

^a Significant differences ($p < 0.05$) from the control based on Dunnett's ANOVA test, assuming normality and equal variance ($p > 0.05$)

^b Significant differences based on Dunnett's ANOVA rank test, not assuming normality and equal variance ($p < 0.05$)

relating to the potential range of impacts that could result from predicted concentrations of atrazine in the surface waters of North America. They concluded that recovery potential of aquatic algae and macrophytes would result in lower impacts on ecological communities observed in the surface water. Similarly, if we consider the ERA of diuron in the Gwydir River catchment, Australia, reported by Burns (2011), it is likely that *L. minor* and *L. gibba* populations exposed to diuron would recover from the effects of a 90th centile 7-day pulse characterized to exceed the hazard concentration affecting 5 % of such species in an species sensitivity distribution (HC_5) ($0.9 \mu\text{g L}^{-1}$), as well as the maximum measured ($77.9 \mu\text{g L}^{-1}$) and predicted ($38.5 \mu\text{g L}^{-1}$) concentrations. In both cases, concentrations of diuron are unlikely to be maintained for a full 7 days. Therefore, the potential for organisms to recover from exposure to different concentrations of pesticides should be more actively considered when conducting catchment-based ERAs. To better predict the ecological effects of agrochemicals, it is recommended that ecotoxicity bioassays attempt to include exposure dynamics likely to be observed in catchments, as well as a recovery phase.

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