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Growth inhibition in early life-stage tests predicts full life-cycle toxicity effects of lead in the freshwater pulmonate snail, *Lymnaea stagnalis*

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ABSTRACT

The freshwater pulmonate snail, *Lymnaea stagnalis*, is the most sensitive freshwater organism tested to date for several metals (Co, Cu, Pb, Ni) based on 28 d early life-stage (ELS) tests in which growth was the most sensitive endpoint. The United States Environmental Protection Agency (USEPA) has expressed concern that growth in 28 d ELS tests with mollusks may overpredict toxicity because of the potential for recovery in a full life-cycle (LC) test. Consequently, the USEPA only accepts the survival endpoint for these tests in establishing water quality criteria (WQC). To address this concern, the current study aimed to test the sensitivity of *L. stagnalis* to Pb in a 56 d full LC test evaluating survival, growth, reproductive and embryonic growth endpoints and compare the estimated effect levels to those established using the 28 d ELS test design. The most sensitive endpoints in this study were 28 d growth and 56 d egg mass production, both with a NOEC of <1.0 $\mu g L^{-1}$ and a LOEC of 1.0 $\mu g L^{-1}$, showing that the ELS growth endpoint is predictive of the 56 d reproduction endpoint. Snails exposed to 1.0 and 2.7 $\mu g L^{-1}$ Pb showed full and partial recovery from growth inhibition between 28 and 56 d. While this recovery supports the USEPA's concern about the 28 d growth endpoint; considering the reproductive lifespan of *L. stagnalis* and the recovery dose-response, we conclude that the 28 d growth endpoint will be within a factor of 3 of full LC endpoints. This is consistent with the level of precision previously determined for fish ELS tests, which the USEPA accepts for WQC derivation, and suggests that tests using 28 d ELS growth endpoint for *L. stagnalis* may be acceptable for inclusion in WQC derivation.

1. Introduction

One of the most significant uses of toxicity testing is to derive water quality criteria (WQC) for the protection of aquatic organisms. In the United States, the Environmental Protection Agency (USEPA) derives both acute and chronic WQC for pollutants. The USEPA defines chronic toxicity tests used to derive chronic WQC by their duration; either as life-cycle (LC) tests, partial life-cycle (PLC) tests, or early life-stage (ELS) tests. These tests are used to determine Species Mean Chronic Values (SMCVs), and the distribution of SMCVs is used to set the chronic WQC (typically at the 5th percentile, which ideally protects 95% of taxa) (Stephen, 1985).

In deriving chronic WQC, the USEPA prefers the use of full life-cycle studies that begin with <24 h old organisms and continue through sexual maturation, reproduction, and typically survival and growth of early life stage F_1 organisms. For fish, full life-cycle studies can require 6–24 months to complete depending on the species. Because of the significant resources required for such studies, the USEPA accepts partial life-cycle (beginning with adult exposures and following through early life stage survival and growth of F_1 fish) and early life-stage (beginning with embryos and following hatching success, survival, and growth through 30 d post-hatch) studies when full life-cycle studies are not available. These shorter test designs for fish have been shown to be reasonably predictive of full life-cycle studies for a wide range of toxicants (McKim, 1977; Macek and Sleight, 1977).

In contrast, for invertebrates, the USEPA has relied almost exclusively on full life-cycle studies for chronic WQC derivation. Full life-cycle studies with invertebrates can typically be performed in ≤60 d and often ≤28 d and are comparable to the level of effort required to perform ELS and PLC studies with fish; however, over the last 25 years, a number of ELS test methods have been developed for invertebrates (i.e., amphipods, aquatic insects, and freshwater mollusks). While there is now a relatively large body of data using the ELS method for invertebrates, there has not been a systematic analysis of how well ELS studies for invertebrates predict results

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Table 1
Chemistry for test water used in 56 d Pb exposure on *L. stagnalis*. Data presented as mean ± SEM.

Parameter	Value
pH	6.89 ± 0.06
Temperature (°C)	24.8 ± 0.2
DO (g L^{-1})	6.86 ± 0.04
[Na^+] (mM)	1.18 ± 0.03
[Ca^{2+}] (mM)	0.69 ± 0.06
[K^+] (mM)	0.03 ± 0.00
[Mg^{2+}] (mM)	0.18 ± 0.01
[Cl^-] (mM)	1.19 ± 0.12
[SO_4^{2-}] (mM)	0.08 ± 0.00
[HCO_3^-] (mM)	0.37 ± 0.06
DOC (μM C)	330 ± 7.02

observed in LC studies. As a result, the USEPA has generally not considered invertebrate ELS data in deriving chronic WQC. This issue was highlighted in the USEPA's recent revision of the ammonia WQC, where the most chronically sensitive freshwater taxa are mollusks based on data from ELS studies (USEPA, 2009). In this case, the USEPA judged that it is unclear whether effects on freshwater mollusk growth in relatively short-term studies (28 d) translate to long-term effects, as these organisms may recover from initial growth inhibition in longer-term (e.g., 90 d) studies. In contrast, survival effects are not reversible; therefore, any observed survival effects during a 28 d exposure are expected to directly predict the outcome of a full life-cycle test if one was performed (USEPA, 2009). Thus, the USEPA judged that in the absence of data from LC studies; survival, but not growth effects, from 28 d invertebrate studies can be used in the derivation of WQC. Other regulatory jurisdictions have less rigorous definitions of what constitutes a chronic test. For example, in the EU, ELS studies with non-standard test organisms, for which there has been no validation of their power to predict LC results, are routinely accepted for purposes of setting water quality guidelines and performing regional risk assessements (Bodar et al., 2005).

Previous studies have established members of the freshwater pulmonate snail genus *Lymnaea* as some of the most sensitive freshwater organisms to dissolved lead (Pb) exposure (Borgmann et al., 1978; Brix et al., 2012; Grosell and Brix, 2009; Grosell et al., 2006b). In particular, the pond snail, *Lymnaea stagnalis*, is the most sensitive freshwater organism tested to date, with an EC20 of <3 μg L^{-1} for growth (Brix et al., 2012). Unlike ammonia, where survival effects occur at concentrations just slightly higher than observed growth effects, no significant effects on snail survival have been observed in these ELS studies at concentrations up to 120 μg L^{-1} Pb.

Consequently, the objective of the current study was to test the sensitivity of *L. stagnalis* to chronic Pb in a full LC exposure, specifically evaluating the USEPA's concern that growth effects observed in previous 28 d ELS studies may not predict effect in full LC studies. Endpoints for this study included survival, growth, and reproduction through 56 d, as well as embryonic growth of F_1 organisms through 10 d of exposure.

2. Materials and methods

2.1. Experimental animals

Adult snails were obtained from an in-house culture maintained in flow-through dechlorinated City of Miami tap water (Table 1). The culture was fed a mix of lettuce and sweet potatoes, and egg masses were transferred from the main culture tanks to static-renewal nursery tanks for hatching before being used in toxicity studies.

2.2. Effect of prolonged Pb exposure on snail weight, specific growth rate, survival and reproduction

A full life-cycle chronic toxicity test (56 d) was performed using newly hatched snails (≤24 h old) in a flow-through system. Dechlorinated Virginia Key tap water was supplied via gravity to a single source container with a volume of 44 L, then to a series of 6 mixing chambers. Total water flow from the source container into the mixing chambers, with volumes of 7 L each, was ~180 mL min^{-1}, where mixing was achieved by vigorous aeration. A constant water level was achieved in the mixing chambers via an overflow drain. Stock solutions of Pb (as $PbNO_3$ in Milli-Q water) were added to the mixing chambers via Mariotte bottles. Each mixing chamber supplied test solution (Pb added) to four replicate 1.5 L test chambers, each containing five newly-hatched snails, at a flow rate of ~8–10 mL min^{-1}.

Prior to test initiation, the flow-through system was operated for 5 d without snails and a small piece of sweet potato was introduced to each exposure chamber. This allowed a biofilm to be established in the exposure beakers upon which the juvenile snails could feed. Snails were exposed to control (<0.18), 1.0 ± 0.16, 2.7 ± 0.38, and 8.4 ± 1.05 μg L^{-1} Pb (measured concentrations). Snail survival was monitored daily. Weekly, snails were blotted dry on paper towels after which total body mass (wet weight) was determined to the nearest 0.1 μg on an analytical balance (Mettler, Toledo). Water samples were collected at the beginning of the experiment and weekly thereafter for measurement of dissolved Pb concentrations (defined as passing through a 0.45 μM filter). In addition, water samples were collected three times weekly for temperature, dissolved oxygen and pH measurements and twice monthly to measure dissolved Na^+, Ca^{2+}, K^+, Mg^{2+}, Cl^-, SO_4^{2-}, and HCO_3^- concentrations. Three samples were collected throughout the experiment for DOC analysis.

Following 32 d of exposure, when the first egg masses appeared in the control treatment, egg masses, if present, were collected daily from each treatment tank and preserved in 10% formalin solution. The number of egg masses per day was counted for each treatment tank. The number of embryos per egg mass was determined on preserved egg masses using a dissecting microscope.

2.3. Embryonic Pb toxicity

Following 56 d of exposure, treatment tanks were cleared of adult snails and egg masses. One egg mass per container was kept and maintained under experimental conditions. Photographs of each egg mass were taken at 4 d and 7 d using a Nikon SMZ800 precision microscope (Nikon Instruments, Melville, NY, USA) equipped with a Firei400 digital camera (Unibrain, San Ramon, CA, USA) under 10×, 15×, and 30× magnification. Egg capsule diameter and snail embryo diameter were determined under 15× magnification using ImageJ software (National Institute of Health, 2011).

2.4. Analytical chemistry

Water samples for determination of Pb exposure concentrations were passed through a 0.45 μm cellulose nitrate syringe filter (Acro-disc, Pall Life Sciences, MI, USA) and acidified by addition of HNO_3 (Fisher Scientific, Trace metal grade) to a final concentration of 1%. Lead concentrations were analyzed by graphite furnace atomic absorption (Varian 220Z, Varian, Walnut Creek, CA, USA) via multiple injection, in which samples were analyzed in duplicate.

Concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} in water samples were determined using atomic absorption spectrophotometry (VarianAA 220FS, Mulgrave, Victory, Australia). Concentrations of Cl^- and SO_4^{2-} were determined using anion chromatography (DIONEX DX 120, Sunnyvale, CA, USA). Concentrations of HCO_3^- were

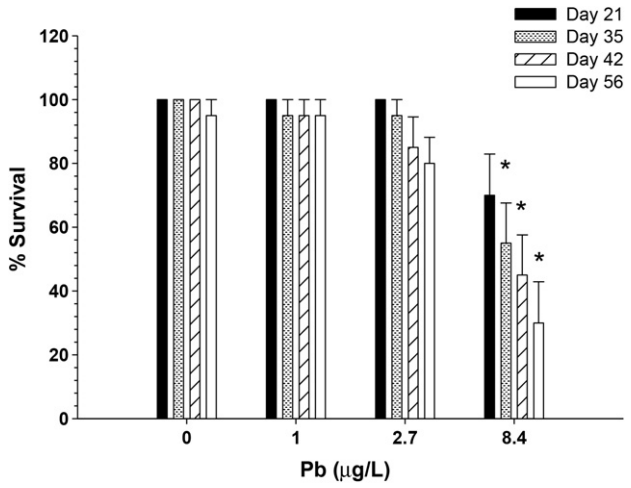

Fig. 1. Survival of *L. stagnalis* over 56 d of Pb exposure. Data presented as mean ± SEM ($n = 4$). "*" indicates statistically significant difference from controls ($p < 0.05$).

determined by double endpoint titrations as in previous studies (Mager et al., 2010). In brief, samples were spurged with N_2 gas for 15 min to rid the sample of dissolved CO_2, pH was recorded, and samples were titrated to a pH of 3.8 using 0.02 N HCl. After being gassed with N_2 for an additional 15 min, samples were titrated back to the original pH using 0.02 N NaOH. The concentration of HCO_3^- in each sample was defined as the amount of HCl subtracted from the amount of NaOH added. Dissolved organic carbon was measured via high-temperature catalytic oxidation using a Shimadzu total organic carbon-VCSH (V series, combustion catalytic oxidation/nondispersive infrared method, Kyoto, Japan). Test water was analyzed for pH using a PHM200 meter (Radiometer, Copenhagen, Denmark) and for temperature and dissolved oxygen using a DO 100 meter (Oakton Instruments, Vernon Hill, IL, USA).

Consistent with Esbaugh et al. (2012), Pb speciation modeling was performed using the freeware program Visual MINTEQ 3.0. The default method settings were used and binding of meal ions to dissolved organic matter (DOM) was modeled using the NICA-Donnan formulation. It was assumed that DOM contained 50% DOC by mass and a composition of 65% active fulvic acid.

2.5. Data analysis

All analyses were performed on measured test concentrations. Data are presented as mean ± SEM with $n = 4$ throughout, unless otherwise stated. Because only three treatments were tested, data were not amenable to regression analysis and were only evaluated using One Way ANOVA tests with a Dunnett post hoc test. Results were considered statistically different at $P < 0.05$. All analyses were performed using SigmaPlot v11.0 (Systat Software, 2008).

3. Results

3.1. Effect of Pb on survival

In the first 21 d of exposure, Pb did not affect survival in any of the three treatments; however, exposure to 8.4 μg L^{-1} Pb resulted in significantly decreased survival during the final 35 d of exposure, resulting in a NOEC of 2.7 μg L^{-1} and a LOEC of 8.4 μg L^{-1} for the survival endpoint (Fig. 1).

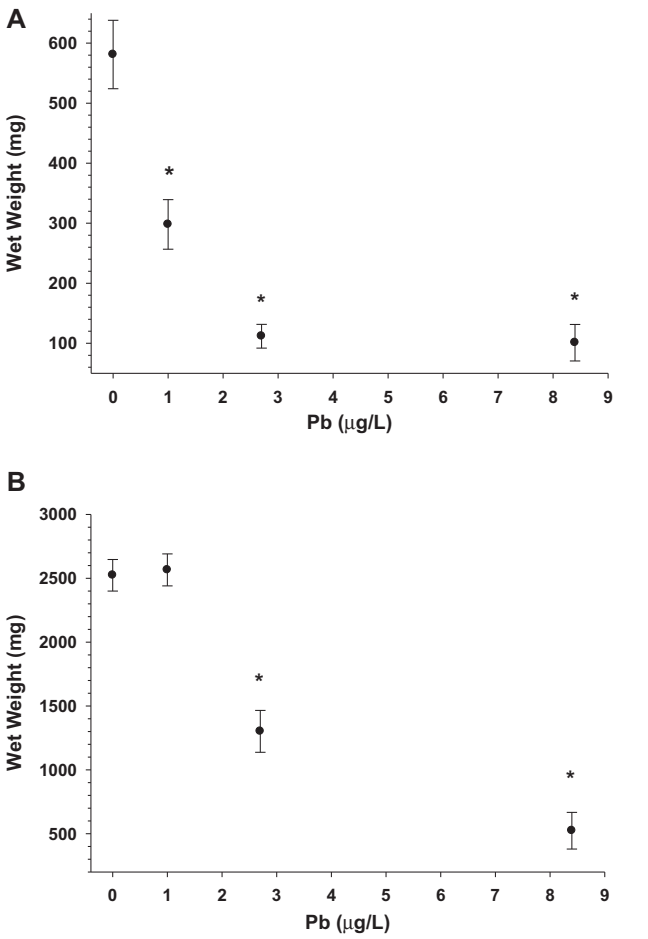

Fig. 2. Effect of Pb exposure on juvenile *L. stagnalis* growth after (A) 28 d and (B) 56 d. Data presented as mean ± SEM ($n = 4$). "*" indicates statistically significant difference from controls ($p < 0.05$).

3.2. Effects of Pb on snail growth

In the first 28 d of exposure, Pb concentration had a significant effect on snail growth (wet weight) at 1.0, 2.7 and 8.4 μg L^{-1}, resulting in a NOEC of <1.0 μg L and a LOEC of 1.0 μg L (Fig. 2A). In the second half of the exposure (28–56 d), snails in the 1.0 μg L^{-1} Pb treatment appeared to recover and only snails exposed to 2.7 and 8.4 μg L^{-1} Pb showed a significant reduction in mass at 56 d, resulting in a NOEC of 1.0 μg L^{-1} and a LOEC of 2.7 μg L^{-1} Pb for growth (Fig. 2B).

Weekly analysis of growth data (as specific growth rate (SGR) relative to the control) shows that snails exposed to 1.0 μg L^{-1} Pb not only recovered, but actually exceeded control growth rates during the final 28 d of exposure (Fig. 3). Additionally, snails exposed to 2.7 μg L^{-1} Pb showed reduced inhibition of SGR in the final four weeks of exposure, with a SGR comparable to the control in the last week of the experiment. In contrast, snails exposed to 8.4 μg L^{-1} showed increased inhibition of SGR in the last 4 weeks of the study compared to the first 4 weeks.

3.3. Effect of Pb on reproduction

During the reproductive phase of the study (from 32 to 56 d), all Pb concentrations were found to have a significant effect on the number of egg masses produced per week per snail, resulting in a NOEC of <1.0 μg L^{-1} and a LOEC of 1.0 μg L^{-1} Pb (Fig. 5). Similarly, all Pb concentrations had a significant effect on the time until the first egg mass in each treatment was produced and on

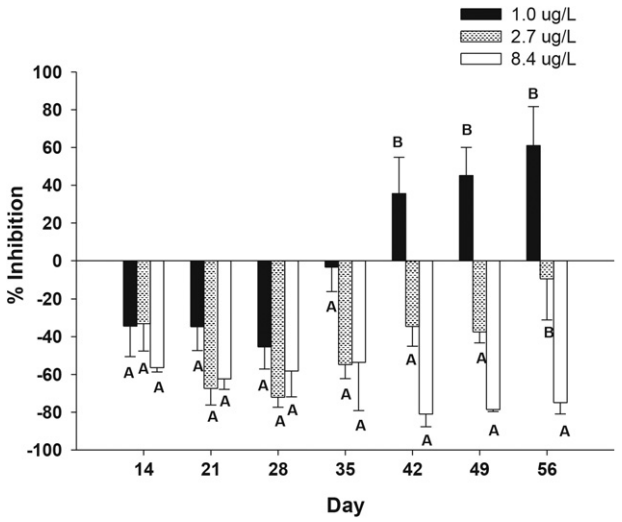

Fig. 3. % Inhibition of specific growth rate of juvenile *L. stagnalis* exposed to 1.0, 2.7 and 8.4 μg L^{-1} Pb. Data presented as mean ± SEM (n = 4). Positive values indicate growth rates higher than controls. Bars with different letters are significantly different (p < 0.05).

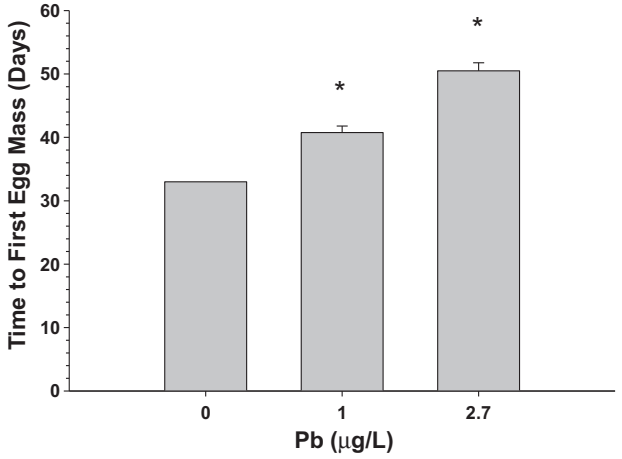

Fig. 4. Effect of Pb exposure on time until first egg mass is produced by adult *L. stagnalis* during the 26 d reproduction period. Data presented as mean ± SEM (n = 4). "*" indicates statistically significant difference from controls (p < 0.05). Note: highest treatment (8.4 μg L^{-1}) did not reproduce during the 24 d period.

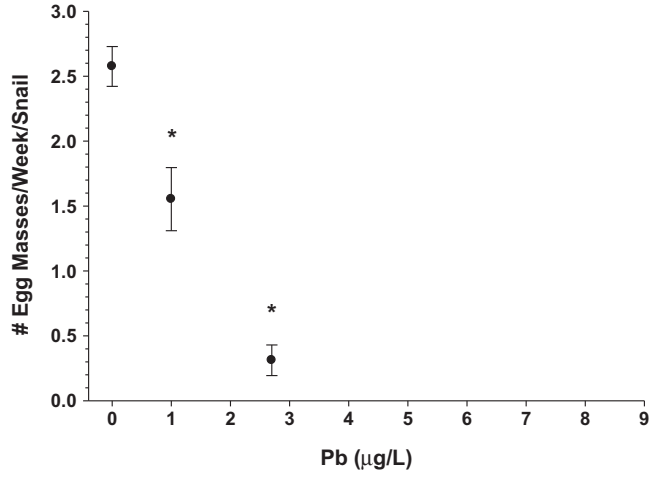

Fig. 5. Effect of Pb exposure on number of egg masses produced per week per snail from 32 to 56 d. Data presented as mean ± SEM (n = 4). "*" indicates statistically significant difference from controls (p < 0.05). Note: highest treatment (8.4 μg L^{-1}) did not reproduce during the 24 d period.

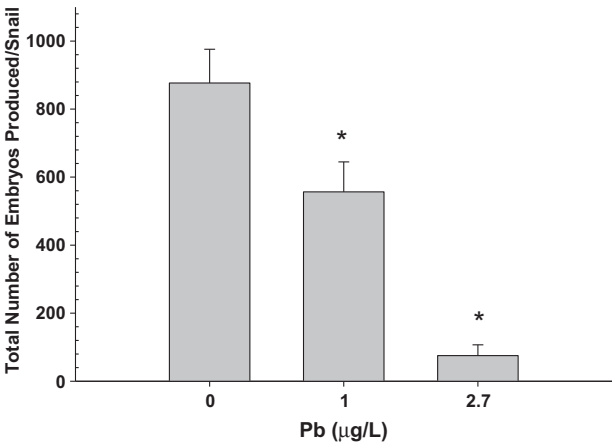

Fig. 6. Effect of Pb exposure on total number of embryos produced per snail from 32 to 56 d. Data presented as mean ± SEM (n = 4). Bars with different letters are statistically different (p < 0.05). Note: highest treatment (8.4 μg L^{-1}) did not reproduce during the 24 d period.

the total number of embryos per treatment per snail, resulting in a NOEC of <1.0 μg L^{-1} and a LOEC of 1.0 μg L^{-1} Pb (Figs. 4 and 6). Snails exposed to 8.4 μg L^{-1} did not reproduce during the reproductive period. Similar to the effects observed on growth, when egg mass production was plotted over time relative to the control, there was a significant, though incomplete recovery trend at 1.0 μg L^{-1} Pb, while at 2.7 μg L^{-1} Pb, a slight statistically insignificant trend toward recovery was observed (Fig. 7). There was no significant effect of the number of embryos per egg mass, with an overall mean number of 76.9 ± 5.19 embryos per egg mass.

3.4. Effect of Pb on embryonic growth

After 4 d of development, Pb significantly affected egg capsule diameter at 1.0 and 2.7 μg L^{-1} Pb; however, at 7 d of development, significant effects on egg capsule diameter were only observed at 2.7 μg L^{-1} (Figs. 8A and 9). Although a general trend of reduction in snail embryo diameter was observed at 4 and 7 d, Pb only had a significant effect on embryo diameter on snails exposed to 2.7 μg L^{-1} following 7 d of development (Fig. 8B).

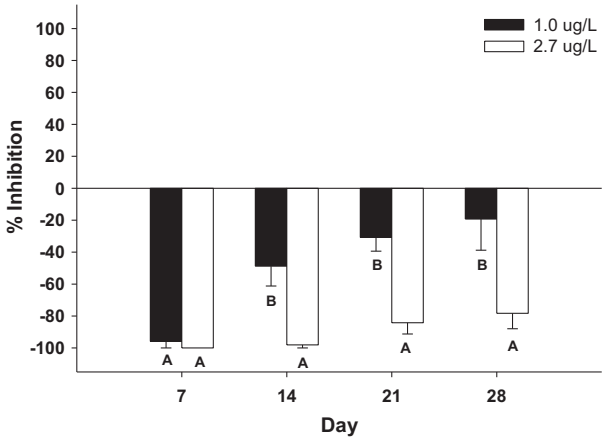

Fig. 7. % Inhibition of reproduction per week of adult *L. stagnalis* exposed to 1.0 and 2.7 and μg L^{-1} Pb. Data presented as mean ± SEM (n = 4). Bars with different letters are significantly different (p < 0.05). Note: highest treatment (8.4 μg L^{-1}) did not reproduce during the 4 week period.

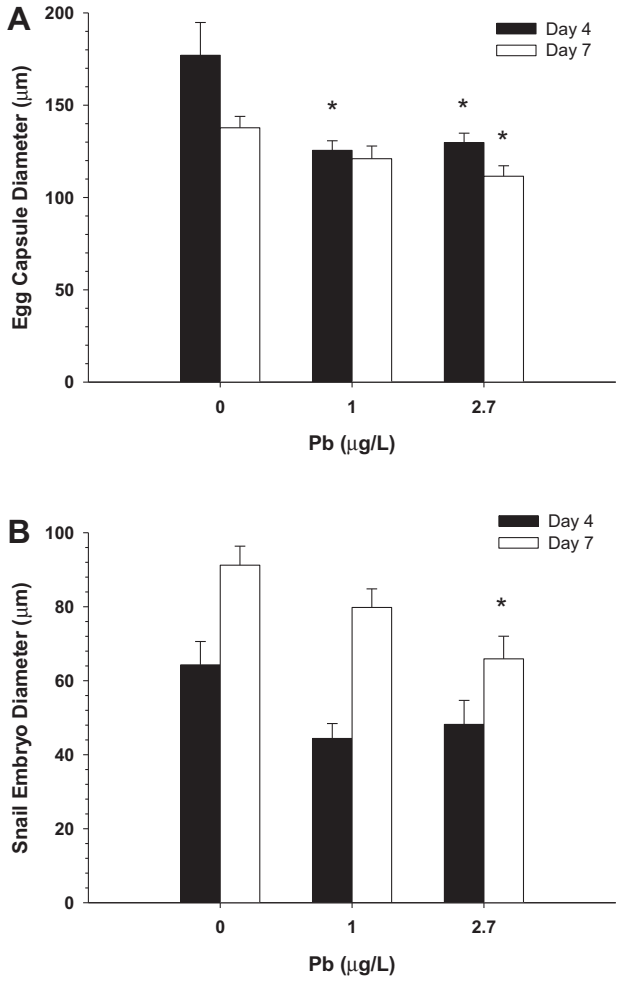

Fig. 8. Effect of continued Pb exposure on (A) egg capsule diameter after 4 and 7 d and (B) embryonic *L. stagnalis* growth after 4 and 7 d egg deposition. Data presented as mean ± SEM ($n = 4$). "*" indicates statistically significant difference from controls ($p < 0.05$). Note: highest treatment (8.4 μg L^{-1}) did not reproduce during the 24 d reproduction period.

3.5. Pb speciation

Pb speciation data using MINTEQ predicted that 99.4%, 99.0%, and 98.3% of total dissolved Pb in the test water was in the form of Pb-DOC in the 0, 2.7 and 8.4 μg L^{-1} treatments, respectively (Table 2).

4. Discussion

The current study sought to characterize the effects of Pb on survival, growth, and reproduction of the freshwater pulmonate snail, *L. stagnalis*, over 56 d of exposure. Previously, we observed survival to be a very insensitive endpoint in a 30 d study with no effects up to 120 μg L^{-1} Pb, ~40-fold higher than the effect level for growth in the same study (Grosell et al., 2006b). In contrast, in the current study, we observed progressively decreasing survival at 8.4 μg L^{-1} Pb, particularly in the last 28 d of the exposure period. This is similar to the effects observed by Borgmann et al. (1978), where progressive survival effects were observed on *L. palustris* such that an asymptotic LC50 was not reached in a 120 d exposure. These effects differ from those observed in larval fathead minnows, where an asymptotic LC50 is observed in 8–10 d (Grosell et al., 2006a).

As in previous 14 and 30 d studies (Grosell et al., 2006b; Esbaugh et al., 2012; Brix et al., 2012), we demonstrated that in a 56 d exposure, snail growth is very sensitive to Pb exposure and *L. stagnalis* is the most sensitive species tested to date for Pb. In the current study, through 28 d, we observed a LOEC of 1.0 μg L^{-1} for snail growth. This effect level is lower than observed in previous studies of similar duration. This is most likely due to the lower pH (7.0) of the dilution water used in this study compared to earlier studies (typically 7.5–8.0), as a reduction in pH will increase Pb bioavailability (Esbaugh et al., 2012). In addition, our Pb speciation data predicted that the majority of Pb was complexed with DOC. Assuming Pb^{2+} is the toxic moiety, the effect level would be 0.004 μg L^{-1} Pb^{2+}. This is within the range of Pb^{2+} EC20s (0.001–0.042) estimated by Esbaugh et al., 2012 for *L. stagnalis* across a variety of different natural waters. As demonstrated by Richards et al., 2001, the extent of protection against Pb toxicity afforded by DOC varies depending on its composition. In the current study, decomposing lettuce was the primary DOC source and the fulvic acid (FA) content of this DOC is currently unknown, leading to some uncertainty in the speciation calculations.

We observed several important patterns in the growth and reproductive endpoints over the course of the study. First, snails exposed to 1.0 and 2.7 μg L^{-1} Pb that had significantly reduced growth rates in the first half of the study recovered during the second half of the exposure. The recovery was incomplete at 2.7 μg L^{-1} Pb. In contrast, at 8.4 μg L^{-1} Pb, there was no evidence of recovery and, in fact, growth rates were progressively inhibited over the entire duration of the exposure (Fig. 3). A similar trend was observed for reproduction, where the number of egg masses per female significantly increased, from the initial inhibition over time at 1.0 μg L^{-1}, and there was at least a slight, though insignificant, trend toward recovery at 2.7 μg L^{-1} Pb (Fig. 7).

We are aware of only two other studies that evaluated freshwater gastropod growth and reproduction over time while exposed to a metal toxicant. In a previous 56 d study with *Lymnaea luteola* exposed to Cu, Das and Khangarot (2011) observed no recovery in either growth or reproduction, but rather an apparent increasing progression of toxicity over time. In contrast, Rogevich et al. (2009) observed an apparent recovery in the apple snail, *Pomacea paludosa* exposed to Cu, with significant effects on SGR at 60 d of exposure, but no effects on SGR after 230 d of exposure.

Despite the suggested recovery of growth in this study, reproduction was still significantly inhibited at the end of the exposure period. Given these limited data for comparison, it is difficult to draw any firm conclusions regarding recovery of snails in full LC studies as compared to ELS-type studies, but we suggest responses may be both species- and metal-specific and that additional studies are needed.

Specific to Pb and *L. stagnalis*, there is clearly evidence of recovery in growth and reproduction in LC studies. At first glance, our data suggests that the USEPA's concern that observed growth effects in standard 30 d studies may not predict growth effects in full LC experiments appears warranted; however, there are a

Table 2
Results of Pb speciation modeling using MINTEQ. Pb-DOC is the sum of FA2-Pb(6) (aq) and FA1-Pb(6) (aq). Mean data is presented as percent of total dissolved Pb.

	Control	2.72 μg Pb/L	8.38 μg Pb/L
Pb^{2+}	0.30	0.40	0.70
FA2-Pb(6) (aq)	93.5	93.8	93.8
$PbOH^{+}$	0.00	0.10	0.10
$PbCl^{+}$	0.00	0.00	0.00
$PbSO_4$ (aq)	0.00	0.00	0.00
$PbCO_3$ (aq)	0.20	0.30	0.50
$PbHCO_3^{+}$	0.10	0.20	0.30
(6)Pb + 2D (aq)	0.00	0.00	0.00
FA1-Pb(6) (aq)	5.90	5.20	4.50
Pb-DOC	99.4	99.0	98.3

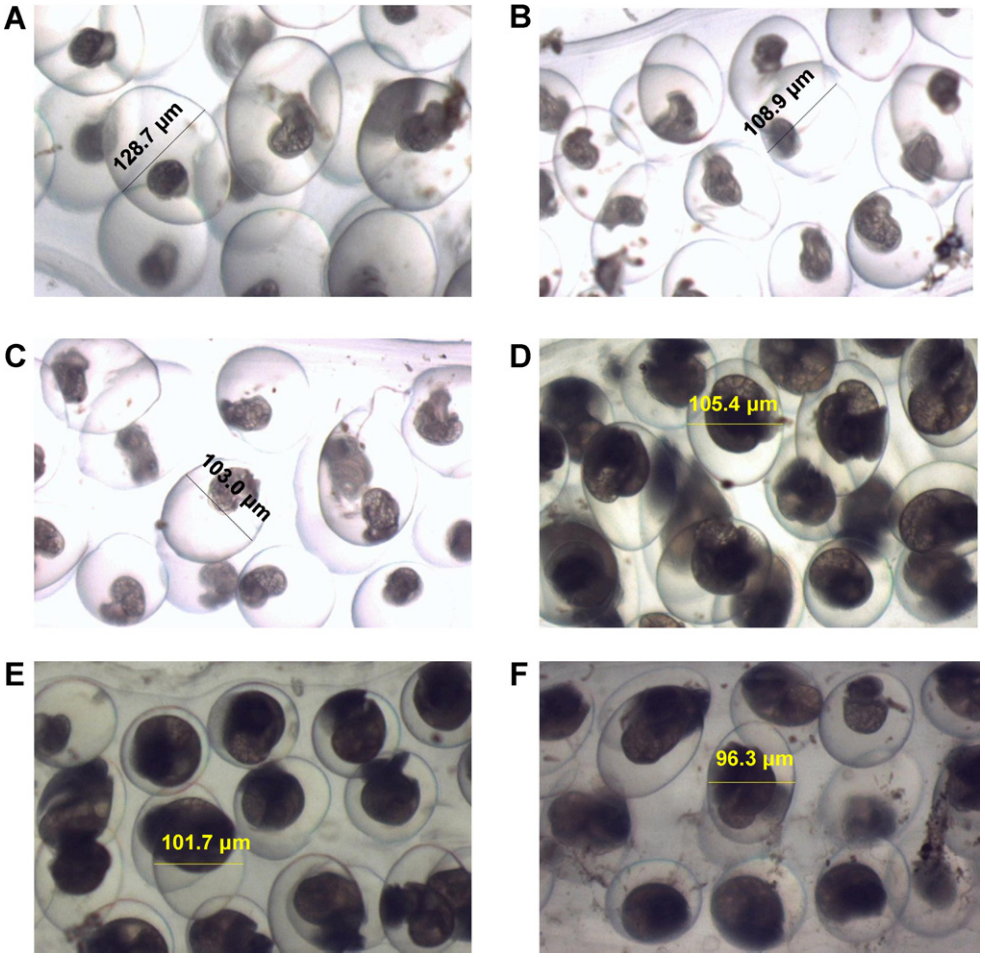

Fig. 9. Egg capsule diameter of representative embryonic *L. stagnalis* after 4 d of exposure to (A) 0 µg L^{-1}, (B) 1.0 µg L^{-1}, and (C) 2.7 µg L^{-1} Pb and 7 d of exposure to (D) 0 µg L^{-1}, (E) 1.0 µg L^{-1}, and (F) 2.7 µg L^{-1} Pb. *Note*: highest treatment (8.4 µg L^{-1}) did not reproduce during the 24 d period.

number of issues to consider. First, it is apparent that *L. stagnalis* growth effects at 28 d are predictive of reproduction effects in a LC study (Fig. 10), although the exact shape of this relationship is likely to change slightly as a function of exposure duration.

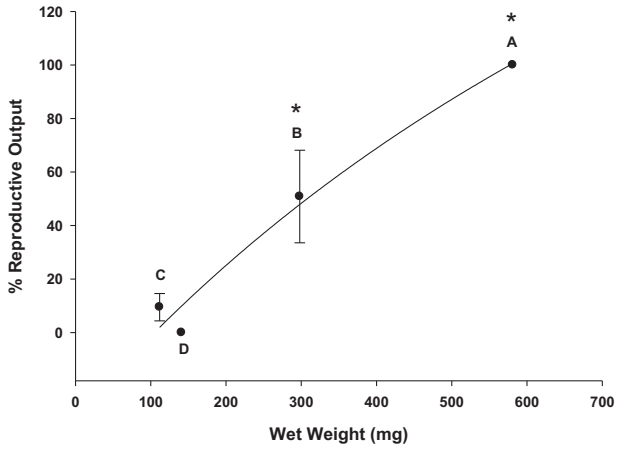

Fig. 10. Relationship between wet weight at day 28 of exposure and subsequent reproductive output of adult *L. stagnalis* exposed to 0, 1.0, 2.7 and 8.4 µg L^{-1} Pb. The equation of the regression line is $y = (1 - 0.0095x)/[-0.0315 - (2.36 \times 10^{-5})x]$, with an *r*-squared value of 0.973. Data presented as mean ± SEM ($n = 4$). Letters indicate exposure treatments, where "A" is 0 µg L^{-1} Pb, "B" is 1.0 µg L^{-1} Pb, "C" is 2.7 µg L^{-1} Pb, and "D" is 8.4 µg L^{-1} Pb. "*" indicates statistically significant difference from controls ($p < 0.05$).

Moreover, based on 28 d growth and 56 d reproduction, the effect level of Pb for *L. stagnalis* differs by a factor of 2.7. *L. stagnalis* has an egg-laying period, under laboratory conditions, lasting between ~85 and 170 d, depending on diet and temperature (Noland and Carriker, 1946). Hence, the 30 d period during which we observed reproductive output in the current study represents a fraction of the total reproductive lifespan of *L. stagnalis* in the wild, although the egg-laying period will likely be shorter in the environment than under controlled, predator-free conditions. While it is possible that reproductive output rates might recover at 2.7 µg L^{-1} Pb if the duration of the study had been extended, given the period of inhibition represents a significant portion of the snail's reproductive lifespan, it is likely that the total lifetime reproductive output would still have been significantly inhibited at this concentration. Similarly, for 1.0 µg L^{-1} Pb, it is unclear if the observed recovery of reproductive output at the end of the study would translate to a full recovery of total life reproductive output.

Given the above, it appears effects on growth observed in 28 d studies are likely to be within a factor of 3 of the effect level (and possibly closer depending on the reproductive recovery trajectory) observed in a full LC study and that any error will be conservative, leading to an overestimation of the effect level. In comparison, for fish, ELS studies are on average within a factor of 1.2 (range = factor of 1–4) of predicting metal toxicity observed in full LC studies, with any discrepancy between the two methods always leading to an underestimation of effect levels (McKim, 1977). Overall, we conclude that this indicates the 28 d ELS study for *L. stagnalis* is comparable (at least for Pb and Cu) to currently accepted fish ELS

studies with respect to predicting full LC effect levels and therefore may be suitable for use in setting WQC. We do, however, note that because *L. stagnalis* is often one of the most sensitive species tested (Grosell et al., 2006b; Brix et al., 2011; Schlekat et al., 2010), it has a large impact on final WQC criteria calculations, such that use of the 56 d LC experimental design described herein may be desirable.

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