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Letters to the Editor

THE HERBICIDE ATRAZINE, ALGAE, AND SNAIL POPULATIONS

To the Editor:

A recent article by Baxter et al. [1] in *Environmental Toxicology and Chemistry* examined the effects of the herbicide atrazine on snail abundance and periphyton (attached algae and food source for snails) in outdoor mesocosms. They concluded that there was no consistent relationship between atrazine and any measured parameter and that this result highlighted the variability in responses of freshwater taxa to atrazine [1]. These conclusions were contrary to previous studies on atrazine, snails, biofilms, and algae [2,3]. They also contradicted review papers concluding that herbicides often indirectly cause algal blooms with subsequent increases in algal grazers [4], and that atrazine has consistent effects on freshwater organisms [5,6].

Inconsistencies across studies can be the source of new ideas that can enhance our understanding of ecological systems, so we examined the Baxter et al. [1] study with much anticipation. However, following scrutiny, we assert that there was probably little statistical power to conclude that atrazine had no effects. In fact, the data have an apparent trend that, if significant, would support, rather than refute, the hypothesis that atrazine exposure is associated with increases in snail populations.

The absence of evidence and the evidence of absence are not equivalent, emphasizing the importance of considering statistical power before concluding that a factor has no effect on a response. Several components of the Baxter et al. [1] study limited their statistical power. First, their experiment only had three replicates of each atrazine concentration. Second, 87% of their replicates had such low dissolved oxygen (DO) concentrations (< 3 mg/L, Baxter et al. Table 3 [1]) that they would be classified as impaired water bodies according to state-level surface water quality criteria of the U.S. Environmental Protection Agency [7]. The power to detect an effect of any contaminant on snails and algae would likely be low under such extreme hypoxic conditions, because these organisms would likely already be near their stress limits. Third, Baxter et al. [1] seemed to analyze their data using ANOVA, which generally provides less statistical power than regression to detect effects of continuous predictors (e.g., atrazine concentration) [8]. However, the exact statistical analyses conducted by Baxter et al. [1] remain equivocal because the authors never provided test statistics (e.g., *F* ratios, chi-square values), probability values, degrees of freedom, or error distributions for any of their statistical tests.

Based on our calculations, a fourth factor that limited the statistical power of the Baxter et al. [1] study was that they overlooked a significant spatial block effect for DO ($F_{2,12} = 5.02$, $p = 0.026$, based on data provided in Table 3 of Baxter et al. [1]). Dissolved oxygen is usually positively correlated with algal abundance because algae release DO, and snail populations are sensitive to both low DO and low algae (e.g., Fox and Taylor [9]). Thus, it is likely that there were at least trends for a spatial block effect on algal and snail populations as well. Overlooking significant variation among blocks can reduce statistical power substantially and conceal significant treatment effects. As an example, consider the hypothetical dataset in Table 1. If we ignore spatial block, there is no significant effect of treatment ($F_{2,6} = 0.49$, $p = 0.6378$), despite treatment A always having larger values than B, and B always having larger values than C within the blocks. If we account for block, the effect of treatment is significant ($F_{2,4} = 49.0$, $p = 0.0015$), and the probability value is reduced 425-fold, emphasizing the potential impact that the missed spatial block could have had on detecting effects of atrazine.

Finally, Baxter et al. [1] did not appear to consider explicitly the effects of atrazine on temporal population dynamics, which also can reduce statistical power and increase the chances of a type II error (a false negative) [10]. It has been hypothesized that atrazine increases snail populations by directly reducing phytoplankton, increasing nutrient and light availability to periphyton, the food source for snails [2]. This mechanism, or others that could be at work, might occur more quickly at high, rather than low, atrazine concentrations because algae die sooner at higher concentrations. This possibility highlights the importance of considering temporal dynamics.

Despite the factors that likely limited Baxter et al.'s [1] statistical power to detect an effect of atrazine, the data they present actually provides a tantalizing trend that might support the hypothesis that atrazine causes alterations in snail populations. For all four atrazine concentrations, snail populations peaked (defined as the first week after week two that a treatment had the highest snail density) before they peaked in the control tanks (Fig. 1), and at the highest concentration, snail populations peaked four weeks before the controls. This pattern was not detected in the analyses ignoring temporal dynamics because all treatments, including the controls, had snail population crashes soon after they peaked (possibly because

Table 1. Hypothetical dataset [a]

Block	Treatment	Response
1	A	11
1	B	10
1	C	9
2	A	8
2	B	6
2	C	5
3	A	5
3	B	3
3	C	2

[a] These data have a presumed normal error distribution that is used to demonstrate the importance of accounting for spatial or temporal blocks.

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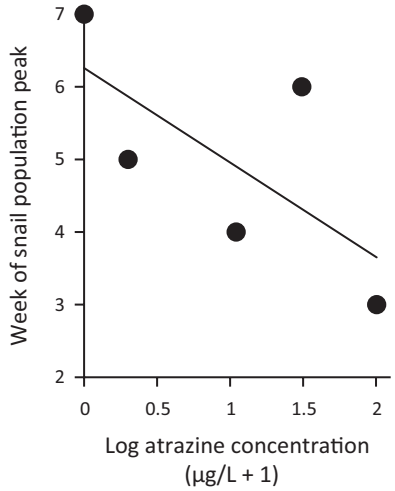

Fig. 1. The relationship between log atrazine concentration and the experimental week that snail populations peaked (defined as the first week after week 2 that a treatment had the highest snail density; $y = -1.3011x + 6.259$, $r^2 = 0.463$). The data were extracted from Figure 5 of Baxter et al. [1].

of temporal dynamics in hypoxia), and thus the effect was transient. Moreover, the apparent relationship between snail population peak and atrazine concentration was generally dose-dependent (Fig. 1), consistent with the hypothesis that higher concentrations of atrazine cause the cascade of events that might fuel snail population growth sooner than lower concentrations.

The only exception to a perfect negative rank correlation between dose and timing of snail peaks was the 30 μg/L concentration (Fig. 1), but this concentration was also the only concentration that did not follow the general pattern of decreasing DO from spatial block 1 to 3 (Table 3 of Baxter et al. [1]), further supporting the notion that the overlooked spatial block was probably important to snail population dynamics. Unfortunately, the statistical significance of this atrazine-associated temporal trend in snail population peaks remains equivocal because we do not have access to the raw data or error estimates. Future studies should test whether this potential relationship between the timing of snail population peaks and atrazine concentration can be reproduced and whether atrazine effects on snail populations depend on algal community composition, water depth, or other factors [11].

In conclusion, Baxter et al.'s [1] experimental design, data, and statistical power do not appear to support their conclusions that there were no effects of atrazine and that their experiment demonstrates high variability in freshwater responses to atrazine. Rather, Baxter et al.'s [1] work might even be consistent with the hypothesis that atrazine exposure accelerates snail population growth and with previous papers showing consistent effects of atrazine on freshwater organisms [2,4–6]. Nevertheless, we commend Baxter et al. [1] for addressing an important and interesting question, and we encourage additional studies on the association between atrazine exposure and freshwater organisms.

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REFERENCES

1. Baxter LR, Moore DL, Sibley PK, Solomon KR, Hanson ML. 2011. Atrazine does not affect algal biomas or snail populations in micrososm communities at environmentally relevant concentrations. *Environ Toxicol Chem* 30:1689–1696.
2. Rohr JR, Schotthoefer AM, Raffel TR, Carrick HJ, Halstead N, Hoverman JT, Johnson CM, Johnson LB, Lieske C, Piwoni MD, Schoff PK, Beasley VR. 2008. Agrochemicals increase trematode infections in a declining amphibian species. *Nature* 455:1235–1239.
3. Staley Z, Rohr JR, Harwood VJ. 2011. Test of direct and indirect effects of agrochemicals on the survival of fecal indicator bacteria. *Appl Environ Microbiol* 77:8765–74.
4. Brock TCM, Lahr J, Van den Brink PJ. 2000. Ecological risks of pesticides in freshwater ecosystems Part 1: Herbicides. Alterra-Rapport 088. Alterra, Green World Research, Alterra, Waginengen, The Netherlands.
5. Rohr JR, McCoy KA. 2010. A qualitative meta-analysis reveals consistent effects of atrazine on freshwater fish and amphibians. *Environ Health Persp* 18:20–32.
6. Rohr JR, McCoy KA. 2010. Preserving environmental health and scientific credibility: A practical guide to reducing conflicts of interest. *Conservation Letters* 3:143–150.
7. Florida Department of Environmental Protection. 2010. *Surface Water Quality Standards* Chapter 62–302. Talahassee, FL, USA.
8. Cottingham KL, Lennon JT, Brown BL. 2005. Knowing when to draw the line: Designing more informative ecological experiments. *Front Ecol Environ* 3:145–152.
9. Fox HM, Taylor AER. 1955. The tolerance of oxygen by aquatic invertebrates. *Proceedings of the Royal Society of London Series B-Biological Sciences* 143:214–225.
10. Rohr JR, Madison DM, Sullivan AM. 2003. On temporal variation and conflicting selection pressures: a test of theory using newts. *Ecology* 84:1816–1826.
11. Rohr JR, Crumrine PW. 2005. Effects of an herbicide and an insecticide on pond community structure and processes. *Ecol Appl* 15:1135–1147.

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The authors' reply:

Several comments have been raised by Rohr et al. regarding our recent publication [1], by means of a letter to the editor of *Environmental Toxicology and Chemistry* [2] and a presentation at the SETAC meeting in Boston, Massachusetts, USA (Paper 419, Nov. 16, 2011). Here, we respond to these comments.

It is correct that our results are different from those reported in Rohr et al. [3] and Staley et al. [4]. While opposing observations may be found for a variety of reasons (further discussed in our paper [1]), we have noted that the Rohr et al. [3] and Staley et al. [4] studies are based on potentially flawed experimental designs. Both microcosm studies [3,4] assessed only a single and unrealistically high concentration of atrazine (nominal of 102 μg/L), derived from GENEEC software, which is not representative of concentrations in ponds in general [5]. GENEEC software is used as a first-tier screening model in the regulatory process and, because it is based on several very conservative assumptions, the estimated environmental con-