# Variation in Pesticide Sensitivity Among Resurrected *Daphnia pulicaria* Genotypes from Hill Lake, MN

**Authors:** Danner Kiernan, Adam Simpson, Dr. Puni Jeyasingh*
Department of Integrative Biology, Oklahoma State University

## Abstract

Previous research on South Center Lake, a eutrophic lake in Minnesota, has shown that ancestral genotypes are more sensitive to a novel contaminant (chlorpyrifos) than contemporary genotypes. It was suggested that the eutrophication history of South Center Lake could have indirectly caused a shift in sensitivity over time. To further investigate this hypothesis, we examined *Daphnia* genotypes originating from a mesotrophic lake in Minnesota (Hill Lake). The genotypes were hatched from dormant eggs that were laid between 1984 and 2010 A.D., which we divided into two age classes: 1984-1997 A.D. (n=3) and 1997-2010 A.D. (n=6). The genotypes were exposed to chlorpyrifos during 48-hour toxicity tests. Sensitivity, measured as the median effect concentration (EC50), was not significantly different between age classes. This lack of a trend could be explained by the lake's trophic status (mesotrophic vs. eutrophic). Further testing of *Daphnia* from Hill Lake could shed light on the influence of eutrophication on contaminant sensitivity.

**Keywords:** Resurrection ecology, chlorpyrifos, eutrophication

## Introduction

Resurrection ecology is the process in which scientists collect sediment cores from the bottom of lakes and hatch preserved resting eggs within the cores. This technique enables scientists to reconstruct the ancient populations of certain species, including *Daphnia*. This is possible because *Daphnia* have unique life cycles. Under normal conditions, *Daphnia* reproduce asexually, creating identical clones of themselves (Miner *et al.* 2012). However, if environmental conditions are poor, they can produce resting eggs, which are laid in protective egg cases (ephippia). Ephippia can become buried beneath the sediment and remain viable for centuries. Therefore, we are able to retrieve eggs from multiple time periods, hatch the eggs, and test the offspring to figure out how the *Daphnia* population has changed over time (Kerfoot *et al.* 2004).

Researchers have found that rapid environmental change can greatly impact the evolution of a species. To test this theory, scientists have used resurrection ecology to reconstruct ancestral population and test how traits have changed over time (Orsini *et al.* 2013). One of the environmental stressors could be eutrophication, or nutrient loading. Eutrophication is an increase in nutrients like nitrogen and phosphorus. This becomes a problem when one of these nutrients, like phosphorus, is present in excess quantities, when they were once a limiting nutrient in the ecosystem. Research has shown that eutrophication can change the physiology of aquatic species by altering metabolic rates (Dillon *et al.* 2010) and changing nutrient use efficiency (Frisch *et al.* 2014). However, it is still unknown how adaptation to eutrophication can affect responses to other stressors, such as pesticides.

The main goal of this study is to better understand how the presence of eutrophication in a lake affects pesticide sensitivity. To test this, we used *D. pulicaria* genotypes that were "resurrected" from Hill Lake, MN, which is classified as mesotrophic (low-intermediate levels of phosphorus). In previous research on a nearby eutrophic lake in Minnesota (South Center Lake), researchers found that ancestral genotypes were 2.7 times more sensitive to chlorpyrifos (an insecticide) than the contemporary genotypes (Simpson *et al.* 2015). This change in sensitivity could potentially be explained by adaptations to a eutrophic environment. Therefore, testing *Daphnia* from a mesotrophic lake (Hill Lake) will allow us to determine how living in a less affected environment affects pesticide sensitivity. The pesticide we use in this study is chlorpyrifos, which allows us to compare our results in Hill Lake to past research done on the same species in South Center Lake.

***Figure 1*** *- The experiment consisted of six treatments (five concentrations of chlorpyrifos, one control), each with three replicates.*

*Table 1 - A comprehensive overview of each genotype from Hill Lake, including chlorpyrifos sensitivity (EC50).*

| Years | Depth | Genotype | EC50 |
|---|---|---|---|
| 1997-2010 | 0-6 cm | 15B | 0.34 |
| | | 3H | 0.25 |
| | | 12H | 0.20 |
| | | Q-8A | 0.34 |
| | | QD-11 | 0.64 |
| | | 9 | 0.48 |
| 1984-1997 | 6-10 cm | 10H | 0.40 |
| | | 99H | 0.46 |
| | | 33 | 0.15 |

## Methods

The *D. pulicaria* genotypes used for this study were hatched from sediment cores collected from Hill Lake, Minnesota in 2011. This study focused on nine genotypes that we separated into two age groups: 1984-1997 A.D. (n = 3) and 1997 – 2010 A.D. (n = 6). The 1984-1997 A.D. genotypes were found in sediment layers 6-10 cm deep in the cores. The 1997-2010 A.D. genotypes were found 0-6 cm deep in the cores (Table 1).

We performed acute toxicity tests for all nine genotypes using the insecticide chlorpyrifos. Chlorpyrifos is a neurotoxic compound that increases action potentials and causes seizures to exposed animals. The reason we chose chlorpyrifos for our toxicity tests was to allow a more direct comparison to the previous study in South Center Lake (Simpson *et al.* 2015), and because it is believed that chlorpyrifos has never been present in Hill Lake. Therefore, it is unlikely that the *Daphnia* have been exposed to the insecticide before.

We began by separating adult females from their stock culture jars into new 1 L jars filled with COMBO culture medium (Kilham *et al.* 2007). We then waited for the *Daphnia* to reproduce asexually. Once they produced enough neonates (babies), we would haphazardly transfer 10 neonates (<48 hours old) into the experimental units. The experimental units were 100 mL beakers filled with 80 mL of COMBO (Figure 1). We prepared six treatments: five concentrations of chlorpyrifos (0.075, 0.15, 0.30,

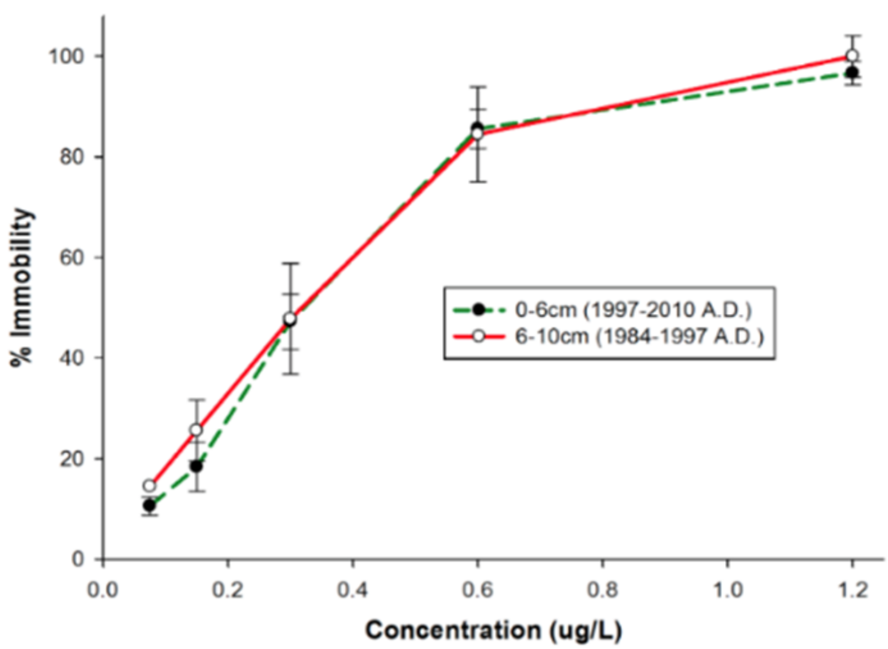


***Figure 2*** *- The dose-response curves of the two age classes from Hill Lake, MN. There is no difference in response (immobility) at each of the five treatment concentrations.*

0.60, and 1.2 ug/L) and a control (methanol). Each treatment had three replicates. Once there was the correct number of neonates in each beaker, we would dose each treatment with 50 uL of each treatment. We would then return exactly 48 hours later and score the test. An individual was scored as "dead/immobilized" if they did not physically respond to prodding. We determined sensitivity by calculating the median effect concentration (EC50) of each genotype (IBM SPSS 20).

## Results

The toxicity tests indicated that there is considerable variation in sensitivity within each age class (Table 1). The most tolerant genotype within Hill Lake belonged to the 1997-2010 A.D. age class, with an EC50 of 0.64 µg/L. The most sensitive genotype belonged to the 1984-1997 A.D. age class, with an EC50 of 0.15 µg/L. Despite all of this variation, there was no difference in mean EC50 for each age class: 1984-1997 A.D. = 0.37 ± 0.16 µg/L, 1997-2010 A.D. = 0.34 ± 0.16 µg/L. Additionally, the percent effect at each treatment concentration did not differ between age classes (Figure 2).

## Discussion

The objective of our study was to determine the toxicity of chlorpyrifos to resurrected Daphnia pulicaria clones from Hill Lake, MN. Our data showed that there is variation in sensitivity among the tested genotypes from Hill Lake. However, there is no difference in sensitivity between the two different age classes (1984-1997 and 1997-2010 A.D.). This differs from the data collected by past researchers who studied the same species in South Center Lake, MN. In South Center Lake, researchers found that ancestral *Daphnia* were significantly more sensitive to chlorpyrifos than the contemporary genotypes (Simpson *et al.* 2015). They hypothesized that the differences in sensitivity were due to differences in evolutionary history. Our experiment might provide some support to that claim, because we tested *Daphnia* from a lake that has not experienced eutrophication. If exposure to eutrophication is causing shifts in sensitivity, then we would expect that the two age classes from Hill Lake would not differ in chlorpyrifos sensitivity, which is what we found. However, we are cautious about making direct comparisons to the study conducted by Simpson *et al.* (2015). Simpson *et al.* (2015) divided the South Center Lake genotypes into three age classes: 1301-1646, 1967-1977, and 2002-2008 A.D. Our study only had two age classes, and both of them were from more recent time periods. This makes it difficult to compare the results of these two studies.

Another thing to consider is that our sample size was relatively low. Unfortunately, we were only able to test nine of the fourteen genotypes from Hill Lake. This was due to issues with culturing. We were unable to successfully culture five of the genotypes, so we could not collect enough neonates to perform tests. In the future, we hope to test all 14 genotypes multiple times to ensure that we discover accurate measures of sensitivity for each age class.

Despite these limitations, our study is still useful in that it provides information about how *Daphnia* populations have changed over time. *Daphnia* are important keystone species in lakes, because they are primary consumers that ensure that algae populations do not grow out of control. They are also non-target organisms of pesticides, so it is important that we understand how they respond to environmental toxicants. Finally, there are few studies that use resurrection ecology to answer questions regarding toxicology, so our experiment adds to this novel line of research. We hope that our results encourage future studies to further investigate how environmental change impacts pesticide sensitivity.

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