EFFECT OF CHLORINE ON MACROINVERTEBRATES IN CROOKED CREEK

Rebecca Bradley, Tanya Dierolf, Ryan Newcomer and Nicole Taibi

ABSTRACT

We tested the effects of sodium hypochlorite on the survival of macroinvertebrates, focusing mainly on the heptageniid mayfly larvae. We hypothesized that higher concentrations of this chemical should cause increased mortality. We found that a significant number of mayfly larvae died in both 2ppm and 4ppm concentrations. In order to further examine this effect, a qualitative and quantitative statistical analysis was performed both upstream and downstream of the effluent from sewage-treatment chlorine beds emptying into Crooked Creek (Huntingdon County, Pennsylvania). Based on our qualitative analyses, we discovered a higher percentage of pollution tolerant macroinvertebrates upstream than downstream, which is not what was expected. Quantitatively, however, more organisms were found upstream, heptageniid mayflies being the most common. Based on our quantitative analyses, we also found that a higher percentage of organisms in the upstream area were pollution intolerant, supporting our hypothesis that water quality was better upstream.

Keywords: abundance, chlorinated sand beds, macroinvertebrates, sodium hypochlorite, species diversity

INTRODUCTION

Macroinvertebrates provide an excellent means to assess stream quality. By observing the abundance and diversity of macroinvertebrates, it is possible to evaluate the presence and intensity of pollution in a stream. We qualitatively and quantitatively analyzed samples from the field paired with the use of a laboratory experiment to study the effect of chlorine on macroinvertebrates.

Disinfectants are used to reduce the load of pathogens in the environment, thus reducing the risk of disease. Chlorine, a popular disinfectant, is highly effective against bacteria and many viruses that cause disease. The township of Hesston, Pennsylvania has employed the use of chlorine beds to treat their sewage as an alternative to traditional sewage treatment. The process begins when the sewage is initially processed into a collecting tank located behind the Hesston post office on Backbone ridge, at the Northeast Extension. The water is then drained into a bacteria treatment tank. The chief treatment of the water commences when the sewage runs through sand beds chlorinated with sodium hypochlorite. Finally the water is channeled to a pipe and released into nearby Crooked Creek.

Chlorination is commonly used as a disinfectant for drinking water at a concentration of 3 parts per million (ppm) and 5 ppm for slime control. However, a study of Crooked Creek by Hayes, McElroy, Skinner, and Skokan (2001) showed that such concentrations could be harmful to the health of non-pathogenic organisms, such as macroinvertebrates. We reproduced this study by qualitatively and quantitatively analyzing 10 random samples of macroinvertebrate abundance and diversity in Crooked Creek both upstream and downstream of the effluent from the chlorine beds. We also tested the effects of different chlorine concentrations on the survival of heptageniid mayflies. We hypothesized that heptageniid survival should decrease with increasing concentrations of sodium hypochlorite.

FIELD SITE

Our field sites were located in Crooked Creek in Huntingdon, Pennsylvania. The source of chlorine output was located in a cul-de-sac off the side of the creek. The upstream area can be described as having small cobble size stones, little vegetation, an island, some shrubbery, shallow to deeper water, and an embankment of 0.5 to 1 feet. The appearance of the downstream area was basically the same except for a fallen tree and lower embankments, about 0.1 to 0.5 feet.

METHODS AND MATERIALS

All sample buckets, containers and glass bowls were washed with deionized water and Alconox Glassware Cleaner in order to prevent contamination. On March 22, 2002, mayflies were collected using kick nets from an area upstream from the source of the sewage-treatment output to ensure that they were not exposed to chlorine. In the laboratory, thirty-six mayfly larvae were exposed to 0, 2 and 4 ppm of sodium hyochlorite (the higher concentrations being in the range actually found in the outputs of the Hesston sewage plant, i.e., 0.5-4 ppm). The concentrations were prepared by taking two 1-gal containers of water and adding 0.65 oz bleach (6% sodium hypochlorite solution) to one and 1.12 oz to the other in order to get 2ppm and 4ppm solutions (following Sanitation-Disinfection Basics 2002; see also Appendix). Two-hundred and seventy ml of upstream creek water was poured into one glass bowl, serving as our control, while 270ml of 2ppm and 4ppm solutions were poured into two other bowls. Twelve mayflies were placed in each bowl and stored in a temperature control unit at 10°C. They were monitored three times a day (10am, 2pm and 6pm) every day for one week in order to record the number of survivors.

On April 3rd and 4th, 2002 we returned to Crooked Creek in order to perform a qualitative and quantitative analysis of the macroinvertebrates upstream and downstream from the chlorine output. We took 10 random samples using kick nets in an area about 30 m from the output. Each sample was collected and emptied into ten separate buckets. The macroinvertebrates were classified into class I, II, and III organisms, which are indicative of different levels of water pollution, as explained further in the Discussion. Taxic richness and diversity were calculated as shown in the Appendix.

RESULTS

The different species were divided into their respective water-quality classifications, I indicating the lowest pollution tolerance and 3 the highest (following Peckarsky et al. 1990). The qualitative analysis revealed a 15.1% greater percentage of class I organisms downstream than upstream (Table 1). However, the quantitative analysis showed that there were 174 more individuals of class I organisms upstream than downstream, resulting in a 28.4% increase (Table 2). Twenty-two macroinvertebrate taxa were collected upstream of the chlorine source, whereas 16 were collected downstream (Table 4). Various taxic diversity measures were also higher upstream than downstream (Table 3).

From the time that the experiment was set up until the time that it was first checked all 12 mayflies had died in the 4-ppm solution (Fig. 1). At the first check there were 3 survivors in the 2-ppm solution and all 12 organisms were still alive in the control experiment. At the time of the second check all the mayflies had died in the 2 and 4-ppm chlorine concentrations. The control organisms did not begin to show a significant death count until the third day.

Location	Class	Total Number of Organisms	Percentage per Class
Upstream	1	13	59.09%
	2	4	18.01%
	3	5	23.00%
Downstream	1	12	75.00%
	2	1	6.25%
	3	3	18.75%

Table 1. Numbers and percentages of macroinvertebrates in three different water-quality categories, both upstream and downstream of the chlorine source in Crooked Creek, Pennsylvania (based on qualitative samples)

Table 2. Numbers and percentages of macroinvertebrates in three different water-quality categories, both upstream and downstream of the chlorine source in Crooked Creek, Pennsylvania (based on quantitative samples)

Location	Class	Total Number of	Percentage per Class
		Organisms	
Upstream	1	334	79.7%
	2	19	4.54%
	3	66	15.76%
Downstream	1	160	51.28%
	2	9	2.88%
	3	143	45.84%

Table 3. Macroinvertebrate species diversity upstream and downstream of chlorine source in Crooked Creek, Pennsylvania

	Simpson's Diversity	y Index	Shannon Diversity Index	
	D value	E value	H value	J value
Crooked Creek				
Up	5.67424	0.25792	2.243436	0.725786
Crooked Creek				
Down	2.870319	0.179395	1.434664031	0.517446

Table 4. Macroinvertebrate taxa collected upstream and downstream of chlorine source in Crooked Creek, Pennsylvania.

Upstream

Decapoda Orconectes (Crayfish) Diptera Emphididae (Red Worm) Coloptera Psephenidae (Water Penny) Trichoptera Brachycentridae (Caddisfly) Ephemeroptera Heptageniidae (Mayflies) Ephemeroptera Ephemeridae (Mayfly) Coloptera Elmidae (riffle beetle) Ephemeroptera Caenidae (Mayfly) Plecoptera Peltoperlidae (Stone Fly) Megaloptera Corydalidae (Helgrammite) Megaloptera Sialidae (Helgrammite) Trichoptera Leptoceridae (Caddisfly) Diptera Chironomidae (midges)

Downstream

Decapoda Orconectes (Crayfish) Diptera Emphididae (Red Worm) Coloptera Psephenidae (Water Penny) Trichoptera Brachycentridae (Caddisfly) Ephemeroptera Heptageniidae (Mayflies) Ephemeroptera Ephemeridae (Mayfly) Coloptera Elmidae (riffle beetle) Ephemeroptera Caenidae (Mayfly) Plecoptera Peltoperlidae (Stone Fly) Megaloptera Corydalidae (Helgrammite) Ephemeroptera Sialidae (Helgrammite) Trichoptera Leptoceridae (Caddisfly)

Isopoda (sowbugs)	Diptera Chironomidae (midges)
Ephemeroptera Baetidae (Mayfly)	Trichoptera Rhyacophilidae (Caddisfly)
Trichoptera Hydropsychidae	Bivalva Sphaerridae
Amphipoda Gammarusminus	
Gastropoda Physidae	
Oligochaeta	
Plecoptera Perlidae	
Nematomorphia (Horsehair worm)	
Anisoptera Libellulidael Coduliidae (Dragonfly Larva)	

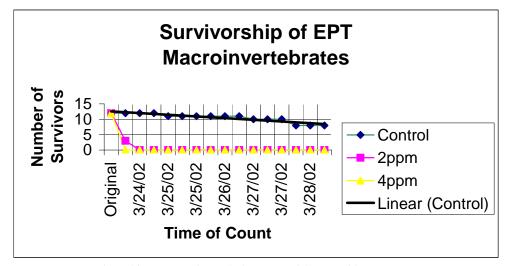


Figure 1. Survivorship of heptageniid mayfly larvae in different chlorine concentrations.

DISCUSSION

We hypothesized that sodium hypochlorite, the chemical used to treat Hesston sewage, would reduce the survival of macroinvertebrates. Indeed, heptageniid mayfly larvae survived less well at higher versus lower chlorine concentrations (Fig. 4). In the time it took for all the larvae in 2 and 4 ppm of chlorine to die off, 100% of the control larvae survived. This experiment should be repeated with replicates so adequate statistical analysis could be carried out.

We supplemented our laboratory study with a field study of the abundance and diversity of macroinvertebrates above and below the chlorine source in Crooked Creek. Both taxic diversity and abundance were greater upstream than downstream (Table 3), suggesting that water quality was higher upstream. Qualitative sampling also revealed a greater number of class I, II, and III organisms upstream than downstream. Class I organisms have little or no tolerance to polluted water and are an indicator of good water quality. Class II organisms rindicate moderate pollution and good to fair water quality. Class III organisms signify poorer water quality and can be found almost anywhere, for such species have a high pollution tolerance (Peckarsky et al. 1990). Of the 22 different organisms found in qualitative sampling upstream, 13, 59%, were class I. Downstream qualitative sampling revealed 12, 75%, of 16 organisms to be class I. Percentages of class II and III organisms were also higher upstream than downstream. More pollution tolerant organisms were observed upstream, than down. This is the opposite of what we expected. Quantitatively, more individuals were found upstream. Heptageniid mayflies, class I, were the most common organism found upstream. Emphidid fly larvae, class III, were the most common organism found upstream. Emphidid fly larvae, class III, were the most common organism found upstream. Thus in contrast to our qualitative results, a much higher percentage of upstream macroinvertebrates were class I organisms than downstream. This does correspond with our hypothesis,

suggesting better water quality upstream from the sewage output pipe. However, all of these trends require more statistical analysis before they can be accepted.

Macroinvertebrates with a smaller body size were also observed upstream. Further research should be conducted to determine whether there really is a body weight/chemical correlation. Smaller organisms may not be able to tolerate high chlorine levels as well as larger organisms. Because downstream macroinvertebrates were larger, it may be possible for higher chlorine concentrations to be distributed at lower concentrations throughout their bodies.

We had also planned on test the chlorine levels of Crooked Creek. However, the necessary chemicals to perform testing were not available due to the time constraints of research. Such testing should help in further evaluating the effects of chlorine on stream macroinvertebrates. Testing water at the pipe would give the chlorine levels that flow directly into the creek. Similar to the experiment performed using heptageniid mayflies, a further experiment could be performed testing the survivorship at those specific parts per million. A transplant study could also be performed. Macroinvertebrates from upstream could be placed downstream, 30 meters from the effluent pipe, and survivorship success could be monitored to see if downstream conditions could be tolerated.

ACKNOWLEDGEMENTS

We thank Dr. Douglas S. Glazier for his help and guidance in completing this study. We also thank Melanie Vrabel, Dr. John Matter and Dr. Charles Yohn for helping us get started. Thanks, too, to Dr. Tom Fisher and Dr. Ei-Ichiro Ochiai for their chemistry expertise and help with the sodium hypochlorite solution. Dr. Paula Martin was helpful with macroinvertebrate identification, as well.

APPENDIX

Sodium Hypochlorite

Chemical Formula - NaOCl

Application - Used as a bleaching agent, oxidant, sterilizer, discoloring agent, deodorant, water treatment, food additives etc. Also used as a food additive.

Properties - Sodium Hypochlorite is a colorless, transparent liquid. It is dissolved in cold water and decomposed by hot water or carbon dioxide. As an excellent chlorine sterilizer, it serves as a strong oxidizer, bleaching agent, and sterilizer. To store Sodium Hypochlorite safely, locate it in a cool, dark place, maintaining pH 11 or more and avoiding mixture with copper or nickel.

Simpson's Diversity Index and Equitability

 $D = 1/\sum_{i=1}^{3} P_i^2$ where S = species richness, and P = the proportion of the total number of individuals (or

biomass) in the community that belong to the i^{th} species.

$$E = \frac{D}{D_{\text{max}}} = \frac{D}{S}$$
 where D_{max} = the maximum equitablity, which = the total number of species in the community (S).

Shannon Diversity Index and Equitablity

$$H = -\sum_{i}^{s} P_{i} \ln P_{i} \text{ and } J = \frac{H}{H}_{Max} = \frac{H}{\ln S}$$

LITERATURE CITED

- Gotelli, N.J. 2001. A Primer of Ecology, 3rd edition. Sinauer Associates, Sunderland, Massachusetts, USA.
- Hayes, D., M. McElroy, E. Skinner and R. Skokan. 2001. Effect of experimental chlorine water treatment beds on macroinvertebrate abundance and diversity in Crooked Creek. *Journal of Ecological Research* 3: 10-13.
- Sanitation-Disinfection Basics. 2002. University of California-Davis. http://www.vmtrc.ucdavis.edu/s_sanitat.html. Accessed March 12, 2002.
- Miller, G.T. 2000. Living in the environment. Brooks/Cole, Albany, New York, USA.
- Peckarsky, B.L., P.R. Fraissinet, M.A. Penton, and D.J. Conklin. 1990. Freshwater macroinvertebrates of Northeastern North America. Cornell University Press, Ithaca, New York, USA.