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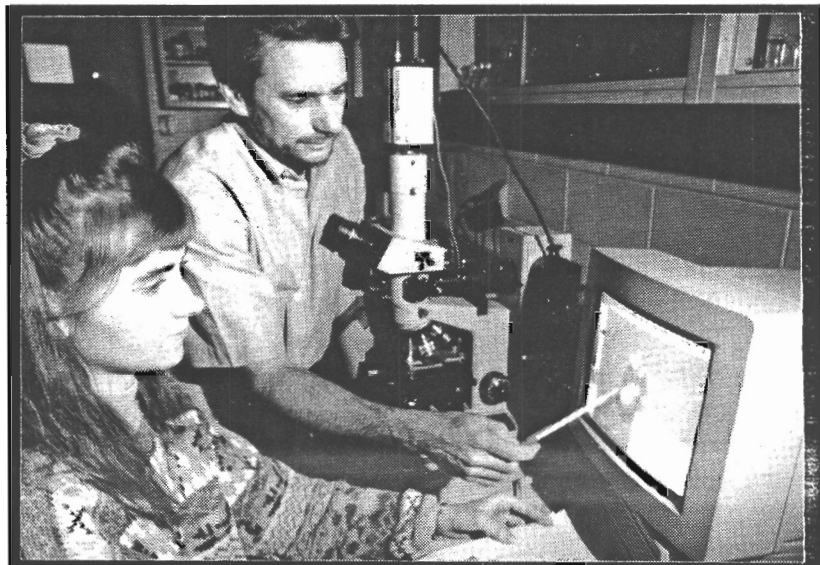
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## GLOW, LITTLE ROTIFER: FLUORESCING ORGANISMS OFFER HELP FOR ASSESSING AQUATIC POLLUTION

A tiny, fluorescing aquatic organism could play a big role in reducing the amount of time and money needed to assess concentrations of pollutants in aquatic environments.

Dr. Terry Snell and colleagues at the Georgia Institute of Technology have measured the concentrations of toxicants in water samples by tracking certain biological activities in rotifers. They will present their findings November 9, 1992 at the Society of Environmental Toxicology and Chemistry annual meeting. The research is funded by the United States Geological Survey, through the Water Resources Institute.

This process is an improvement over the usual



*Dr. Terry Snell, right, points out fluorescence in a rotifer to graduate student Charlotte Juchelka. Color Slides/B&W Prints Available*

toxicology testing methods of exposing microscopic animals to polluted water samples and then tracking their mortality rates, or studying how their reproduction is inhibited, Snell said. Measuring rotifers' ingestion rates and enzyme activities provides results faster and less expensively, he explained.

"Typically, it takes four to seven days to get the results back on a test of reproduction inhibition," said Snell,

associate professor of biology. "A reproductive test with cladocera, small aquatic crustaceans, is going to cost \$1,000 if you do it commercially. We can do one of these rapid tests using enzyme activity or ingestion rates for about 50 times less because less technician time is needed."

The tests offer potential for doing more than just

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assessing the toxicity of aquatic environments, Snell noted.

"Animals, especially aquatic animals, are being exposed to many manmade stresses," he explained. "If we are concerned about preserving biodiversity, we need to understand how animals respond to stress. In understanding this response, we might be able to find ways to reduce the effects of stress."

The multicellular aquatic animals used in these experiments, rotifers, are known for the wheel-like ring of cilia on their anterior ends. They are about 1/32nd of an inch long, transparent, and barely visible to the naked eye. About 10 could fit on the head of a pin. These tiny herbivores eat unicellular freshwater and marine algae, recycle nutrients and are prey for larval shrimp, fish and crabs. The researchers chose rotifers because they are small, have short life cycles, and are easy to culture in a laboratory.

In their study of ingestion rates, the researchers exposed the rotifers to samples of water with varying concentrations of the toxicants pentachlorophenol, copper and cadmium. The animals then were exposed to fluorescently labelled latex spheres, which they ingested. The fluorescing of the spheres inside the animals could be viewed by the researchers using a microscope equipped for epifluorescence.

Findings indicated that ingestion rates were significantly reduced as toxicant concentrations increased. The fluorescence of rotifers exposed to water with as little as 20 parts per billion or 20 mg/liter of copper in it, for example, dropped from 160 fluorescence units in controls to almost zero fluorescence units.

The ingestion rate test run for 15-minute exposures was not quite as sensitive as a 48-hour reproduction test, but was equal to or more sensitive than mortality tests. The sensitivity of the ingestion test could be increased with longer exposure times. In addition, the researchers are exploring the use of ingestion rates to predict how reproductive rate might be affected later on.

The enzyme activities Snell and his colleagues chose to monitor were those of esterase and phospholipase, which are used in digestion, detoxification and other cellular processes. In this study, the rotifers were exposed to a sample of water containing a toxicant, and then were exposed to edible substrates that fluoresce when they are cleaved

apart by the enzymes being monitored.

The researchers have found a strong relationship between toxicant concentration and enzyme activity, which is substantially reduced at higher concentrations of toxicants. The esterase activity in the rotifers' guts, for example, dropped five-fold as concentrations of pentachlorophenol increased from 1 to 2 mg/L. The enzyme activity test was found to be less sensitive than the reproduction tests, but about as sensitive as mortality indicators.

The researchers' objective is to develop faster, more cost-effective toxicity tests while retaining sensitivity, technical simplicity and ecological relevancy, Snell said. This will allow the use of toxicity tests to expand into new applications and be used with relative ease.

In the future, Snell would like to use a gene from fireflies to produce transgenic fish, rotifers, shrimp and other animals that actually light up when they are unstressed. As the animals become stressed by toxicants, their bioluminescence would get dimmer and dimmer, Snell said.

"We build in what is called a reporter system, where the animal indicates to us automatically how much stress it is experiencing by the amount of light it is producing," he said. "This is better, because fluorescence is based on only one enzyme. Bioluminescence is based on cellular ATP levels, which are a more general indicator of cellular stress."

Other professors participating in this research project are biology professor David Dusenbery; associate professors of biology Lloyd Dunn and Nancy Walls; and assistant professor of chemistry Ken Busch. Participating students include graduates Susan Burbank and Charlotte Juchelka; and undergraduates Marcus Vogt, Mike Blakeney and Traci Battle.

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