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Staff Report

Clam-Rotenone Bioassay
October 1-11, 1985

A clam-rotenone bioassay was undertaken in response to concerns expressed by Michigan Department of Natural Resources (MDNR) staff over the possible effects of rotenone on certain rare or endangered species of clams inhabiting streams proposed for fish reclamation projects.

Findings and Conclusions

1. Clam mortality of long term consequences was not measured at rotenone concentrations slightly above that used in normal fish reclamation projects. No mortality was observed during the first 24 hours of the recovery period.

2. Clam mortality, five days after a 10 hour exposure to 1.25 mg/l of rotenone, was 8% (one of 13 clams) at ≈68° F and 15% (two of 13) at ≈75° F.

3. Clam mortality, five days after a ten hour exposure to 2.5 mg/l rotenone (2.5 x normal fish reclamation projects), was 17% (two of 12) at ≈68° F. At ≈75° F an air stone was left out of the dish the second night after exposure and 75% (nine of 12) clams were dead the following morning.

4. No clam glochidia were observed after rotenone exposure.

5. Villosa fabalis (state endangered)might be more sensitive to rotenone than either V. iris or Elliptio dilatata.

Methods

Three species of clams, Villosa iris, V. fabalis (state endangered) and Elliptio dilatata were selected for exposure to rotenone at approximate common project conditions (8-10 hrs. exposure at 1.0 mg/l or parts per million) and at about twice the usual rotenone conditions. Two water temperatures ≈68° F (20° C) and ≈75° F (24° C) were selected for exposures which would approximate the diurnal range of summer water temperature fluctuations. Rotenone exposures were carried out at somewhat

higher temperatures initially but the recovery temperatures were +75° F. Rotenone concentrations in the exposures were 1.25 mg/l (1x) and 2.50 mg/l (2x). Mortality was measured 5 and 9 days after exposure.

Clams were collected from the Pine River in St. Clair County on September 29, 1985 by Leni Wilsmann of the Michigan Natural Features Inventory. Water temperature at the time of collection was 55° F. Clams were transported and held in a cooler with water at 51° F. Clams were received on September 30, 1985 in excellent condition and transported to the Surface Water Quality Division's Bioassay Laboratory. Water temperature in the cooler had risen to 59° F. An air stone was placed in the cooler water at that time and left overnight to allow water temperature to reach ambient room temperature 68° F.

The following day clams were segregated into six dishes (10-13 per dish), each containing two liters of aerated activated carbon-filtered Lansing City water. Three dishes were placed in a water bath where water temperature was gradually raised to the test temperature (75° F). The remaining three dishes were left on the table next to the water bath. A bubbling stone was placed in each dish to maintain oxygen concentrations near saturation. Acclimation to these conditions was extended for another 24 hours.

The next day rotenone was added to four of the six dishes to give concentrations of rotenone of 1.25 and 2.50 mg/l at each test temperature. After ten hours exposure, which would approximate exposures under field conditions, the clams and containers were thoroughly rinsed, filled again with two liters of conditioned water, replaced in the water bath or left on the table as appropriate, for nine additional days before the experiment was terminated.

Rotenone used for test dilutions was a 2.5% solution, labelled as Nusyn-Noxifish, Fish Toxicant synergized rotenone, liquid-emulsifiable.

Observations of the clams were made several times each work day during the acclimation period, during exposure and following exposure. Clams that were agape or failed to respond to stimulation were considered dead. Water temperatures were routinely measured. Water chemistry was run on the rotenone test dishes at the end of the exposure period for conductivity, pH, dissolved oxygen, alkalinity and hardness. At the end of the ten hour exposure period, an aliquot of water was taken from each dish and observed under the dissecting microscope for glochidia that might have been released due to test exposures or conditions.

Results and Discussions

The design and results of this clam-rotenone bioassay are shown in Table 1. No mortality occurred in the tests or controls at either

68° F or 75° F during the ten hour exposure nor was any observed within the following 24 hours.

Five days after exposure to 1.25 mg/l rotenone, only three deaths occurred among the 26 exposed clams (12%). One of six (16%) Elliptio dilatata at 68° F died and two of four (50%) Villosa fabalis at 75° F died. No glochidia were observed in the water at the end of the exposure period.

Five days after exposure to 2.50 mg/l of rotenone, two deaths resulted at 68° F (one of four V. fabalis and one of six E. dilatata). Nine of twelve clams were dead in the 75° F treatment by the fifth day following exposure. This was primarily caused by conditions during the second night following exposure when the air stone was not in the dish for an unknown time period. All V. fabalis (4) and V. iris (2) died while only three of six E. dilatata died under these extreme conditions. The results of this part of the experiment should not be used to estimate the effects of a 10 hour exposure to 2.5 mg/l rotenone at 75° F.

No mortality was observed the day following exposure in any of the treatments, although some indication of stress, as indicated by the presence of slime-foam, was evident in the 2.5 mg/l exposures. Ten of twelve clams at 68° F and at 2.5 mg/l rotenone exposure did recover from the stress and lived to the end of the experiment. This suggests that similar survival, except for V. fabalis, would have occurred at 75° F in the 2.5 mg/l exposure if the air stone had been in the dish at all times. Villosa fabalis, the smallest of the tested species, may be more sensitive than the others to temperature and rotenone. Half of the specimens of V. fabalis (two out of four) died at the 1.25 mg/l exposure at 75° F while none of the others died.

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Table 1. Experimental Design and Results for the Clam-Rotenone Bioassay, October 2-11, 1985.

	Water Temperature ~68°F (20°C)	Water Temperature ~75°F (24°C)
Controls	Shell length in cm	Shell length in cm
V. fabalis	1.0,1.1,1.5	1.8,2.1,2.3
V. iris	4.7,5.7	4.6,5.1
E. dilatata	4.6,5.0,5.5,5.7,5.8	4.5,5.9,6.3,6.7,7.2
Exposure 1.25 mg/l (1x)		
V. fabalis	1.6,2.0,2.2,2.8	1.7,1.9*,1.9*,2.5
V. iris	3.8,5.1,5.7	3.9,4.2,4.7
E. dilatata	5.0*,6.4,6.5,6.8,7.5	4.9,5.6,5.7,6.5,6.6,6.7
Exposure 2.50 mg/l (2x)		
V. fabalis	1.7,1.8*,2.7,3.2	1.7*,1.7*,2.1*,2.7*
V. iris	5.7,6.0	5.0*,5.4*
E. dilatata	5.3,5.6*,5.9,6.7,6.8,6.9	4.9,5.3*,5.5,5.7*,5.7*,6.4
		air stone not in dish (2x ~75°F) the night of October 3, 1985.

No mortality October 3, 1985 during the first 24 hours of the recovery period.
*dead on October 7, 1985, no additional mortality on October 11, 1985.