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## THE USE OF METHYLENE BLUE TO REVIVE WARM-WATER FISH POISONED BY ROTENONE

Gerald R. Bouck and Robert C. Ball Department of Fisheries and Wildlife, Michigan State University East Lansing, Michigan

FREQUENTLY it is desirable to revive fish after poisoning with rotenone, or necessary to assure that the toxic influence of rotenone in rivers will not pass a certain point. The use of permanganate to oxidize rotenone has met with varying degrees of success, but has not been of value in reviving fish. Thus, we noted with great interest that Oberg (1961) and Lindahl and Oberg (1961) had found that rotenone blocked the transfer of electrons in the mitochondria and, further, that treatment with methylene blue reduced this respiratory inhibition. This information gave promise that fish poisoned by retenone could be revived and later used for re-stocking, and that rotenone could be used with a greater degree of safety in rivers.

Three separate occasions allowed the preliminary investigation of the use of methylene blue in reviving fish poisoned by rotenone. Each time methylene blue was used as an emergency measure, and these instances did not lend themselves to experimentation or quantification, but the results were so successful that a preliminary report appears justified.

In the first instance, a farm pond was poisoned with rotenone to get rid of carp, bullheads, and creek chubs. Fish were collected as they surfaced and were placed mamediately in a 15-quart pail containing

approximately 2 milliliters of 5 percent methylene blue in rotenone-free water. As soon as the pail was filled with fish, it was emptied into approximately 150 gallons of rotenone-free water that contained enough methylene blue to color the water strongly. After about 270 fish (2 to 20 inches in size) had been collected, this tank was transported to the laboratory. The fish were then transferred to three 250-gallon tanks filled with tap water containing methylene blue. On the following day, these tanks were drained and refilled with aged tap water. No further treatments were given.

Each day the tanks were inspected and cleaned and the dead specimens were recorded. At the end of 10 days, only 11 of the fish had died and the others appeared to have recovered fully. In view of the crowded conditions and the traumatic experience, this survival was considered significant: handling losses alone could have accounted for this mortality. The fish remaining in the poisoned pond did not receive any methylene blue, and the kill seemed to be complete.

The second opportunity to use methylene blue occurred without warning while a stream was being poisoned to obtain fish biomass estimates. A 530-foot section of a small warm-water river (45 cubic feet per second) was treated with a calculated rotenone concentration of 0.5 p.p.m. for 1 hour. At the downstream end of this section, potassium permanganate solution was bled into the river, beginning with the leading edge of the water mass. Although

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this amount was more than twice that estimated as being required to oxidize the rotenone, it was apparent that the rotenone was still toxic beyond the calculated terminal area of the experiment.

To relieve the effects of the rotenone, about 0.25 pound of methylene blue was added to the water mass containing rotenone. From the point where methylene blue was added to the river and throughout the downstream area, a recovery was effected. Except for a few fish that were drifting into the area treated with methylene blue, no fish were observed to be in distress in this area. Continued observations confirmed that the kill had stopped at the point where we added methylene blue.

A third experience with methylene blue as an emergency antidote occurred during a pollution-induced fish kill. Here the lack of game-fish species restricted our treatment to carp. Immobilized specimens were collected, transported in river water, and placed in aged tap water in the laboratory. Methylene blue was added to half of these (separate) fish tanks. Although both lots ultimately recovered, the treated fish appeared to have made a complete recovery in 12 hours, whereas the untreated fish showed effects of the poison for 24 hours.

An attempt was made to determine the optimum concentration of methylene blue for use in a 3-hour treatment. Rock bass and rainbow trout were exposed to 0.5 p.p.m. rotenone at 16° C. in aquaria. Rainbow trout were affected immediately and could not be revived by treatment with

methylene blue. At this temperature, the rock bass were not affected strongly until 20 minutes had passed, after which they were transferred to aged tap water containing various concentrations of methylene blue. At or above a concentration of 5 p.p.m. of methylene blue, all the rock bass survived. All fish which were not treated with methylene blue died within 24 hours.

Some caution is necessary in the use of methylene blue. Although it apparently is not toxic to fish, it can kill higher aquatic plants. Also, methylene blue encourages bacterial growth on fish. This can be corrected by following the methylene blue treatment with a 1-p.p.m. aureomycin bath for 2 to 3 hours. Methylene blue did not revive rainbow trout poisoned by rotenone, and may not be effective on other cold-water fish. It should not be used where it will stain boats, docks, or other public property.

## Literature Cited

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Observations indicate that coronetfishes use large leopard groupers as shields to stalk prey. Obviously, the coronetfishes do not consider the groupers as predators, and the prey of the coronetfish do not fear the groupers. A comparison of stomach contents has shown that these species have no food in common except the herring.

To minimize the sinking of plastic drift cards, they are now being sealed individually with a small heat sealer and water-tested to detect imperfections.