RESPIRATORY STRESS IN YELLOW PERCH INDUCED BY SUBTOXIC CONCENTRATIONS OF DIQUAT[1]

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Abstract. Two-year-old yellow perch (*Perca flavescens*) from Chautauqua Lake in western New York State were examined using short-term static bioassay techniques to determine possible deleterious subtoxic effects of Diquat (1,1'-ethylene-2,2'-dipyridylium dibromide), an aquatic herbicide commonly used in the lake. Under laboratory conditions, a significant level of respiratory stress was suffered by fish at Diquat concentrations similar to those applied to the lake during weed control measures and at concentrations significantly lower than those causing death (1 to 5 ppm).

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Diquat has been applied to Chautauqua Lake in varying amounts (Nicholson and Rosenthal, 1974) since its initial use as an aquatic herbicide in 1965. Published information available on fish tolerance to Diquat and most other aquatic herbicides is usually expressed in terms of LD–50's, median tolerance limits, and other similar toxicological evaluations dealing only with acute mortality. Determination of lethal concentration end point provides little or no information on the subtoxic effects of Diquat on fish.

In fish, direct contact between the aquatic environment and the gill epithelium may cause these surfaces to become sensitive to environmental alteration in the presence of toxic materials or other irritants. Of special interest was the "cough" response (Schaumburg *et al.*, 1967), a detectable reversal of water flow over the gills. In our study, respiratory pressure changes, caused by the expansion and contraction of the buccal and opercular cavities during the respiratory cycle of the fish, were monitored by buccal catheterization prior to and after exposure to Diquat in aquaria maintained in environmental chambers.

METHODS AND MATERIALS

Two-year-old yellow perch, aged by scale analysis, were seined from an untreated area of upper Chautauqua Lake at depths of up to one meter. The fish were then transported in aerated containers to the laboratory and maintained in epoxy-coated plywood holding tanks. Experiments were performed within two weeks of capture.

The fish were anaesthetized in a solution of 100 mg/*l* of tricaine methanesulphonate and, in an operation taking no longer than 60–90 seconds, a buccal cavity catheter (polyethylene tubing, 0.023″ I.D., 0.038″ O.D.) was implanted posterior and medial to the external nares using the techniques recommended by Schaumburg *et al.* (1967), with minor alterations. Instead of a single length of tubing between the fish and transducer, a short (2–3 cm) length of tubing was initially implanted into the buccal cavity. This section could be sealed off and the fish, unrestrained, could be allowed a greater degree of freedom during the post-operative recovery period; also permitting a greater flexibility in experimental procedures. Prior to each experiment, the fish were lightly anaesthetized, and the buccal catheter was connected to an intermediate length of tubing, utilizing a stainless steel coupling. The opposing end of the tubing was then connected to a sensitive pressure transducer and component amplifying and recording systems.

Static bioassays (Brungs, 1973; Doudoroff *et al.*, 1952; Stephan and Mount, 1973) were conducted in 20-liter aquaria in an environmental chamber at 17°C (lake temperature during the period of experimentation) with oxygen levels maintained close to saturation (>90%). After transfer to a testing aquarium, the fish were allowed sufficient time (48 hours minimum) to recover from stress incurred during handling (Wells, 1932). After the period of acclimation, a 24-hour continuous recording was taken to determine individual baseline data. Diquat was then introduced via a remote system of tubing in an attempt to minimize disturbance to the subject. After a one-hour delay, recording

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commenced. Continuous recordings of the ensuing cough responsiveness of the fish over periods of 48–72 hours to 1 and 5 ppm Diquat were made. In these recordings, normal pressure changes were eliminated by decreasing the sensitivity of the amplifier, thereby allowing only the more forceful cough responses to be recorded. Ten trials were run; three at 5 ppm, five at 1 ppm, and two controls were also monitored. One ppm Diquat is the estimated dosage commonly applied to the lake during weed control operations with local concentrations of up to 10 ppm found immediately after spraying (Mayer, personal communication).

RESULTS AND DISCUSSION

Cough frequency data, at six-hour intervals, were statistically examined (0.05 level of significance) and compared to baseline information for each individual and the results are shown in figure 1.The data indicate that in every instance, upon exposure to Diquat, statistically significant increases in the cough frequency occurred at both concentrations of Diquat used in this study. The controls and

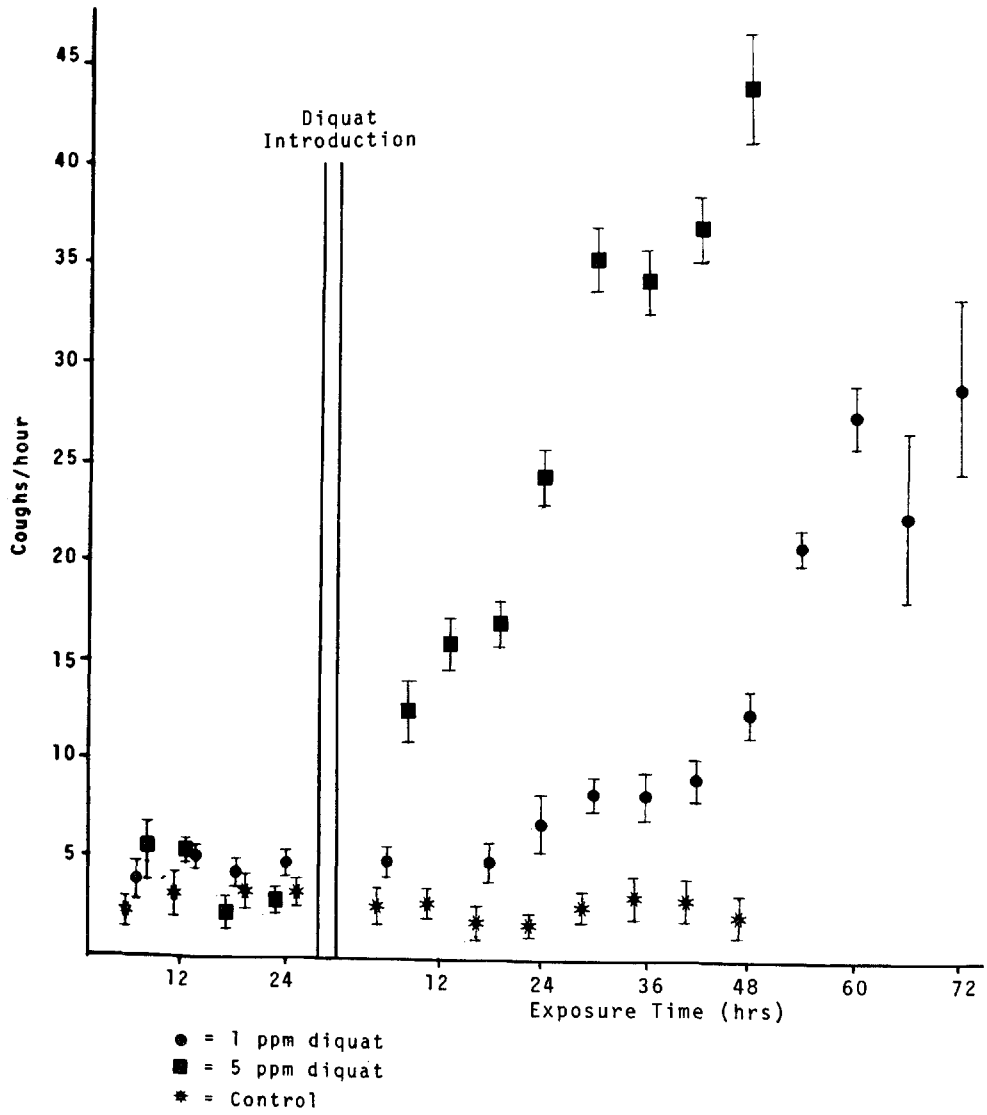

FIGURE 1. Mean cough response of fish before and after exposure to Diquat.

test fish monitored during the 24-hour baseline period exhibited no statistically significant differences in cough rate.

The techniques and the use of respiratory stress to monitor sublethal effects of intoxication were previously applied to a variety of toxicants and subjects with similar results (Schaumburg *et al.*, 1967; Davis, 1973; Walden *et al.*, 1970). Factors including individual sensitivity and species variation (Drummond *et al.*, 1973) and the physical environment (Wilson and Bond, 1969; Yeo, 1967; Walker, 1964) may influence the relative toxicity of Diquat in an aquatic system.

Little is known about the mechanisms involved in the physiological damage to the respiratory apparatus of fish exposed to toxic environments. Respiratory irregularities are thought to be caused by mucous precipitation on the gill epithelium in response to a toxicant (Schaumburg *et al.*, 1967). This may result in a decrease in the dissolved oxygen at the gill surface, initiating the cough reflex which is an attempt to cleanse the respiratory surfaces. Tissue hypoxia of the gill has been found to be a major factor in the deaths of fish subjected to toxic levels of heavy metals (Burton *et al.*, 1972; Skidmore, 1970). The effectiveness of the gill epithelium, not only as an organ of respiration, but as the mediator of osmoregulation and associated processes may be severely impaired by sublethal quantities of toxic substances. Further investigation will be necessary to delineate the extent of damage during exposure to Diquat and the direct influence upon survival, growth, and reproductive success.

Evaluation of toxic chemicals applied to aquatic systems by chronic bioassay to determine lethal concentrations and to define acceptable limits of application are designed to minimize damage to aquatic organisms. It is doubtful that this type of bioassay actually accomplishes the complete task. Subtoxic bioassay should also be incorporated to provide another tool for toxicological evaluations. Although our data are not voluminous, they clearly indicate that a significant level of respiratory stress was suffered by yellow perch during exposure to Diquat at concentrations equivalent to those applied to Chautauqua Lake during weed control measures.

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