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The Effect of Lethal Copper Solutions on the Behavior of Rainbow Trout, *Salmo gairdneri*

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Abstract. Experiments were performed in flow-through artificial stream apparatus to determine the attraction-avoidance responses of rainbow trout, *Salmo gairdneri* to lethal copper solutions (0.5, 0.75, 1.0, 2.0, 3.0 and 4.0 mg/L) when food was provided at a single source in the more contaminated water and when food was provided throughout the stream. Preference of groups of twenty test individuals for the experimental or control water was recorded at hourly intervals over 96 hr. At all concentrations, there was an initial attraction period for and subsequent avoidance of the more highly contaminated waters. Attraction was greatest in tests employing higher concentrations (3.0 and 4.0 mg/L); this attraction led to high mortality. Belated avoidance of copper solutions was observed at all levels above 0.5 mg/L maximizing at 1.0 mg/L. The EC50-96-hr value for avoidance was between 0.5 and 0.75 mg/L. With food provided only in contaminated water, fish exhibited a prolonged and intense attraction period at the lower levels (0.5 to 2.0 mg/L) when compared to dispersed feeding. No significant differences between the feeding regimes existed at the higher copper levels. Mortality curves were similar in both feeding routines. Injured fish were found in the least contaminated waters. Tagging experiments indicate a movement pattern initially toward the toxicant, with subsequent reverse migration to cleaner waters. Little movement occurred during attraction and avoidance stages. Initial avoidance was not observed at any of the tested copper concentrations. Results indicate initial attraction at all concentrations may orientate fish toward the contaminants source, and subsequent avoidance behavior had little effect on survivorship rates. Our results indicate that observed trout behavior subsequent to copper discharges contributed to high mortality. The results also suggest that behavioral response of organisms to toxicants must be incorporated into work attempting to set reasonable water-quality standards in natural water bodies.

Attraction to or avoidance of harmful water is a means of evaluating an aquatic organism's success in surviving an environmental perturbation. Kleerekoper (1976) discussed the importance of studying the behavioral effects of sublethal concentrations of pollutants. The dispersal and subsequent dilution of a pollutant from its source seems a reasonable argument for concentrating studies on low, subacute levels rather than lethal. However, it has recently been reported that high dosages of certain toxicants, such as copper, attract certain species (Black and Birge 1980; Giattina *et al.* 1982). If increasing attraction correlates with increasing toxicant concentration levels, fish may subject themselves to toxic levels at the source of the contamination in areas where random sampling procedures indicate sublethal levels. Therefore, when establishing water-quality criteria the preference or avoidance of fish to potential toxicants must be considered. Furthermore, the behavior of fish and other species must be taken in to consideration when deciding methodology of controlling toxic spills and preserving the aquatic ecosystem.

Behavioral responses to copper, one of the most toxic of heavy metals commonly found in natural waters, differs with concentration and species (Spear and Pierce 1979). Sprague (1964) reported avoidance of 9.0, 28.8 and 90.0 µg/L of dissolved copper by Atlantic salmon. Both attraction (300–

390 μg/L) and avoidance (10-200 μg/L) of rainbow trout to copper was reported by Giattina *et al.* (1982).

Geckler *et al.* (1976) established that laboratory and field tests to determine toxicity of organisms to copper were comparable; however, copper toxicity was underestimated because avoidance-attraction responses were not included in the laboratory tests. A knowledge of behavior responses of fish subjected to a range of copper concentrations could be beneficial for the environmental planning of aquatic ecosystems. If organisms are attracted to toxic discharges, confinement of highly contaminated areas could dramatically increase survivorship of these organisms, hence reduce deleterious effects of these discharges.

Our experiments were prompted in large part by observations of trout behavior following accidental copper discharges into a New Brunswick river (Gwillym 1984). Discharges contained copper concentrations of 1.0-2.0 mg/L, clearly lethal to trout species (Brown *et al.* 1974; Spear and Anderson 1975), while the reservoir into which the river flowed had copper concentrations of about 0.1 mg/L. Nonetheless, schools of trout congregated at the mouth of the river, resulting in extremely high mortality of trout (J. D. Parkin, pers. comm.). We were curious to see if trout would react to such a fairly steep gradient in laboratory conditions as reported by Giattina *et al.* (1982).

Hence, the present study was designed to determine the influence of copper upon a "school" of fish given the choice between differing copper concentrations. Rainbow trout was selected because of its importance in the commercial and recreational fisheries in southern Quebec, Ontario and the Maritimes.

The objectives of this study were to: (1) determine how attraction and/or avoidance vary with time and concentration upon fish populations, (2) if there is avoidance, determine the lowest effective concentration of copper whereby 50% of the fish population avoid the copper solutions (96-hr EC50), (3) observe if the presence of higher concentrations of copper would lead to increased avoidance and thus greater survivorship, and (4) determine how attraction-avoidance responses differ when feeding is used as a stimulus for the test species to enter higher contaminated waters.

Materials and Methods

Juvenile rainbow trout, *Salmo gairdneri* were obtained from La Pisciculture Mont Sutton, Sutton, Quebec. Test organisms (mean length 15 cm) were held under continuous flow conditions in

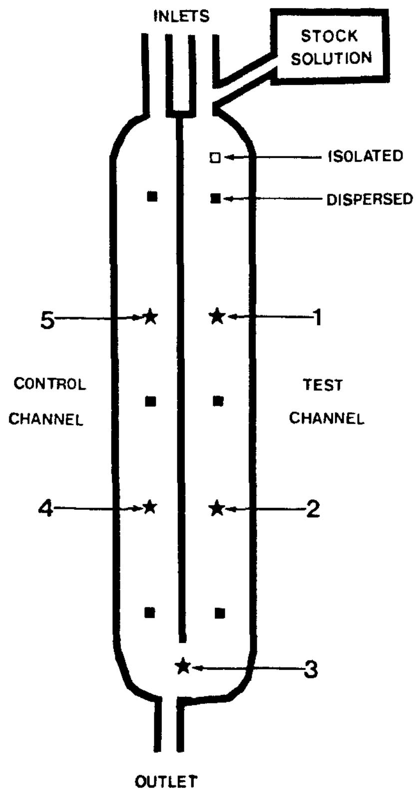

Fig. 1. Diagram of the experimental apparatus. Numbered stars represent water sampling areas. White box denotes isolated feeding, black boxes, dispersed

dechlorinated Montreal city water (12.5 ± 1.0°C) with the following characteristics: pH = 7.2, mean alkalinity = 80.0 mg/L $CaCO_3$, total hardness = 122.0 mg/L. The fish were fed trout chow at a level of approximately 1% of their wet body weight/day, while subjected to a photoperiod of 12 hr light, 12 hr dark.

Artificial streams (250 × 60 × 30 cm high) constructed of polyvinyl chloride (PVC) were divided equally into two sections (Figure 1) such that one side would contain highly toxic water compared to the second side. Dye tests showed a sharp gradient between stream sides. A plastic mesh covered the stream apparatus. Intramedic polyethylene tubing (ID = 0.86 mm) was used to provide a constant flow of 1.6 ml/min from a stock solution. Inflow of dilution water was regulated on both sides at 3.2 L/min for a 99% replacement time of 1.5 hr as calculated from Leduc (1966). Copper solutions (0.5, 0.75, 1.0, 2.0, 3.0, and 4.0 mg/L) prepared with $CuSO_4$ with 0.1 ml/L concentrated nitric acid (HNO_3), were delivered to one side of the stream. Drainage occurred at an undivided end of the streams, thereby providing test specimens with a choice of waters.

Two separate tests were performed, one with feeding only occurring at the source of the treated water (isolated feeding), the other with dispersed feeding on both sides of the stream (Figure 1). A longer (144 hr) test was later performed with isolated feeding at 0.5 mg/L copper concentration. Groups of twenty fish were introduced into the streams and allowed a two-day orientation period. During controls and under test conditions, the number of individuals in the separate waters was visually recorded in a ten minute observation period every hour. All injuries (indicated by unbalanced swimming and "coughing" like motions) were recorded along with mortalities. Dead fish were

Table 1. Measured copper concentrations (mg/L) with standard deviation for stream areas (see Figure 1) in all experiments. Areas 1 and 2 representing toxified stream side with areas 4 and 5 representing cleaner waters

	Areas				
Feeding	1	2	3	4	5
Control					
Isolated	<.05	<.05	<.05	<.05	<.05
Dispersed	<.05	<.05	<.05	<.05	<.05
Experiment					
1A Isolated	0.50 ± .03	0.50 ± .02	0.33 ± .05	0.05 ± .01	<.05
B Dispersed	0.50 ± .05	0.52 ± .03	0.36 ± .01	0.11 ± .01	0.08 ± .04
2A Isolated	0.76 ± .03	0.76 ± .04	0.58 ± .07	0.07 ± .03	0.06 ± .02
B Dispersed	0.78 ± .08	0.77 ± .10	0.52 ± .06	0.10 ± .04	0.07 ± .02
3A Isolated	1.03 ± .08	1.01 ± .08	0.90 ± .09	0.09 ± .07	0.09 ± .07
B Dispersed	1.03 ± .10	1.02 ± .10	0.77 ± .04	0.09 ± .02	0.09 ± .07
4A Isolated	1.96 ± .04	1.98 ± .03	1.58 ± .08	0.25 ± .08	0.22 ± .07
B Dispersed	1.99 ± .02	2.05 ± .03	1.54 ± .02	0.20 ± .03	0.17 ± .03
5A Isolated	3.06 ± .20	2.99 ± .07	2.39 ± .26	0.10 ± .00	0.19 ± .13
B Dispersed	3.00 ± .08	3.08 ± .06	2.28 ± .11	0.19 ± .13	0.11 ± .01
6A Isolated	4.08 ± .08	4.05 ± .04	3.11 ± .13	0.32 ± .04	0.30 ± .03
B Dispersed	4.20 ± .08	4.10 ± .10	3.16 ± .40	0.32 ± .03	0.35 ± .08

removed. No deaths were recorded in any control experiments. All experiments persisted 96 hr, unless 100% mortality occurred before that time. Location of fish in the streams was analyzed by the Run's Test (Zar 1974) to determine if fish were randomly distributed in the apparatus. Experiments were performed in Feb and March 1984.

Total copper concentrations was determined by flame atomic absorption spectrophotometry (Perkin-Elmer Model 503) from 25 ml samples taken twice daily at four depths from five sample areas (see Figure 1) over the duration of the experiments. The mean copper concentration in all water samples is summarized in Table 1.

Results

The percentage of fish found in the more highly contaminated water as a function of time is shown in Figure 2. Data were analyzed and found to depart from randomness by a Run's test (Zar 1974) in all cases except the controls. Controls indicate that, in the absence of copper, fish had no preference for either side of the stream, even during isolated feeding. In all experiments there was an initial attraction period. At lower concentrations (0.75, 1.0 and 2.0 mg/L), water preference changed dramatically over the course of the assay. In isolated feeding experiments performed at 1.0 mg/L, 72.0% of the test species preferred contaminated waters at 24 hr, while at 72 hr only 10.0% of the species preferred the same waters. Similar responses were observed at 0.75 mg/L level. At 2.0 mg/L the range went from 84.75% preference at 6 hr to 15.8% at 24 hr. Similar fluctuations were recorded at these levels with dispersed feeding. Tests at the higher concentrations (3.0 and 4.0 mg/L) gave similar results, but mortality was high (Figure 3), hence avoidance occurred with so few fish that results can not be considered absolutely conclusive.

At the lowest concentration (0.5 mg/L) tested, there was a different response between feeding routines. There was an initial attraction period which gave way to slight avoidance (approximately 40%) after 48 hr in dispersed feeding experiments. Avoidance did not occur with isolated feeding, where the initial attraction period persisted throughout most of the test period, but decreased to no preference by the end of the study. A longer test period (144 hr) confirmed avoidance behavior after 96 hr.

The estimation of an avoidance EC50 would have to take into consideration that 50% of the population would normally occupy that side of the streams. Therefore 50% avoidance (or attraction) does not exist until under 25% of the test species were present on the concerned side. The lowest level where avoidance exists was at 0.75 mg/L; therefore, the EC50 lies between 0.5 and 0.75 mg/L (Figure 2). Initial attraction to copper seems to peak at 2.0 mg/L with a relatively more prolonged effect at higher concentrations (Figure 3).

Tagging experiments indicates that there are distinct attraction and avoidance periods. During attraction phases, fish congregated themselves under the toxic inflow. Meanwhile, during avoidance, test individuals were displaced evenly throughout the control channel.

Mortality curves (Figure 4) show similar death rates between feeding routines, with the exception of 2.0 mg/L concentrations. A more prominent

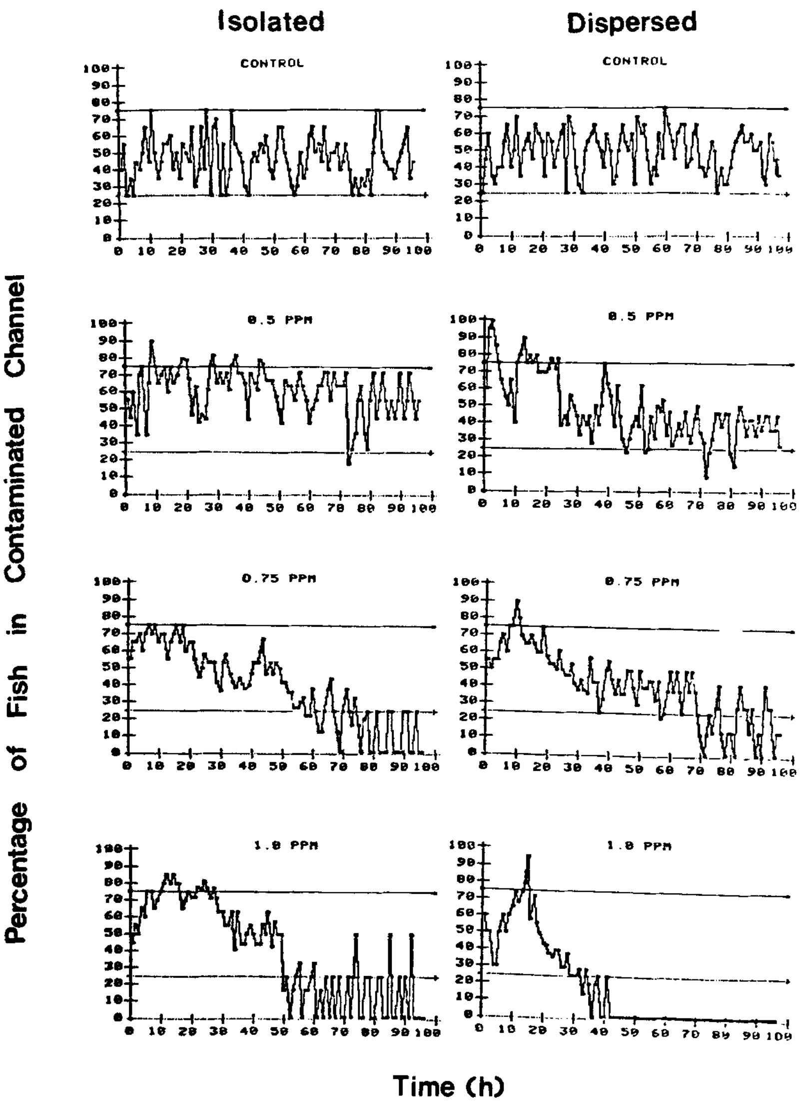

Fig. 2. Percentage of fish located in the more contaminated water over time at copper concentrations between background levels and 1.0 mg/L. Horizontal line at 75% represents attraction, at 25% avoidance. Isolated represents a feeding regime only in the more toxic water, while dispersed represents feeding throughout the stream apparatus (Figure 1)

early attraction response promoted 100% mortality within 28 hr with isolated feeding, while total mortality did not exist until the 64th hour with dispersed feeding, where there was a lesser degree of initial attraction. Injured fish were almost always found at the lower copper concentration (Figure 5).

Discussion

This work investigated the attraction-avoidance behavior of rainbow trout provoked by copper contamination. The behavioral response of fish toward the copper contamination is crucial when attempting to control the repercussions of this toxic metal.

The vast majority of behavioral tests (Giattina *et al.* 1982; Kleerekoper *et al.* 1972; Sprague 1964; Westlake *et al.* 1974) involve short-term observations of single organisms subjected to sublethal toxic levels. Experimentation here indicates short-term elevated attraction to copper at all levels above 0.5 mg/L with subsequent avoidance varying on concentration. Substantial avoidance (75%) at 0.5 mg/L does not occur enough to be considered tangible. Nevertheless, this level killed 50% of the test species by the end of the test period (96 hr). At 0.75 mg/L, avoidance exists, but, as in higher concentrations, not until an initial attraction period leads to high mortality. Giattina *et al.* (1982) estimated an avoidance threshold limit for rainbow trout of 6.4 μg/L, while finding a strong attraction response to lethal levels (330.0 and 390.0 μg/L). Black and Birge (1980) reported that trout avoided low concentrations of copper (0.07 mg/L) while higher levels (4.56 mg/L) resulted in significant attraction. Work done on behavior responses with low copper concentrations (Giattina *et al.* 1982,

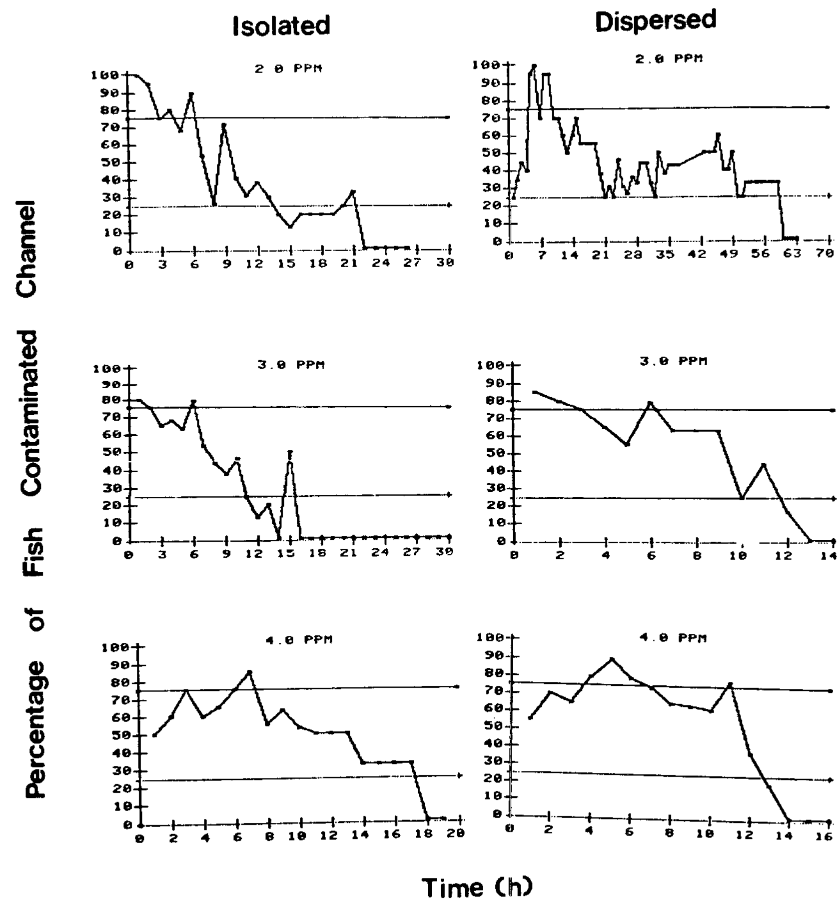

Fig. 3. Percentage of fish located in the more contaminated water over time at copper concentrations between 2 and 4 mg/L

Kleerekoper *et al.* 1972) indicates that there is attraction at trace levels, possibly due to physiological requirements. Hence, it appears that there is a limited range of copper concentrations which fish avoid.

Results based upon short-term monitoring do not take into consideration possible effects of long-term exposure such as acclimation and behaviour response to the toxicant in darkness (Cripe 1979). Our findings with groups of test individuals rather than single individuals indicate that in nature "schools" of fish may be more attracted by copper solutions than previously believed. Initial preference for lethal copper concentrations may cause considerable mortality.

The artificial stream apparatus provided a sharp gradient between ordinary and modified waters, unlike that which one would encounter in nature. However, Giattina *et al.* (1982) found no differences in avoidance threshold levels of rainbow trout to copper when exposed in shallow and steep gradients. Ishio (1965) suggested that behavior in toxic solutions depended on the gradient of the infiltration as well as concentration. Other authors (Kleerekoper *et al.* 1972; Westlake *et al.* 1974) have reported orientation differences toward changing gradients, however, this has only been recognized with low levels of copper (less than 50 μg/L) and with certain species; goldfish (*Carassius auratus*) and channel catfish (*Ictalurus punctatus*). While certain species such as the largemouth bass (*Micropterus salmoides*) showed no specific orientation behavior toward the different gradients (Timms *et al.* 1972). Since Giattina and his co-workers (1982) reported no significant orientation differences by rainbow trout toward shallow and steep copper gradients concerning concentrations up to 0.4 mg/L, we agree with their thought that, due to the ease of quantifying and analyzing the response, a steep gradient would seem more appropriate method for determining behavioral responses.

Furthermore, our experimental design; long-term monitoring, 12 hr photoperiod and usage of groups of test individuals, rather than single individuals, related this study more to natural conditions than previous studies. Geckler *et al.* (1976) reported avoidance of a number of fish species (stiped shiner, rainbow darter, creek chub, fantail darter, sunfish and bluntnose minnow) toward copper concentrations up to 120 μg/L in a controlled contamination

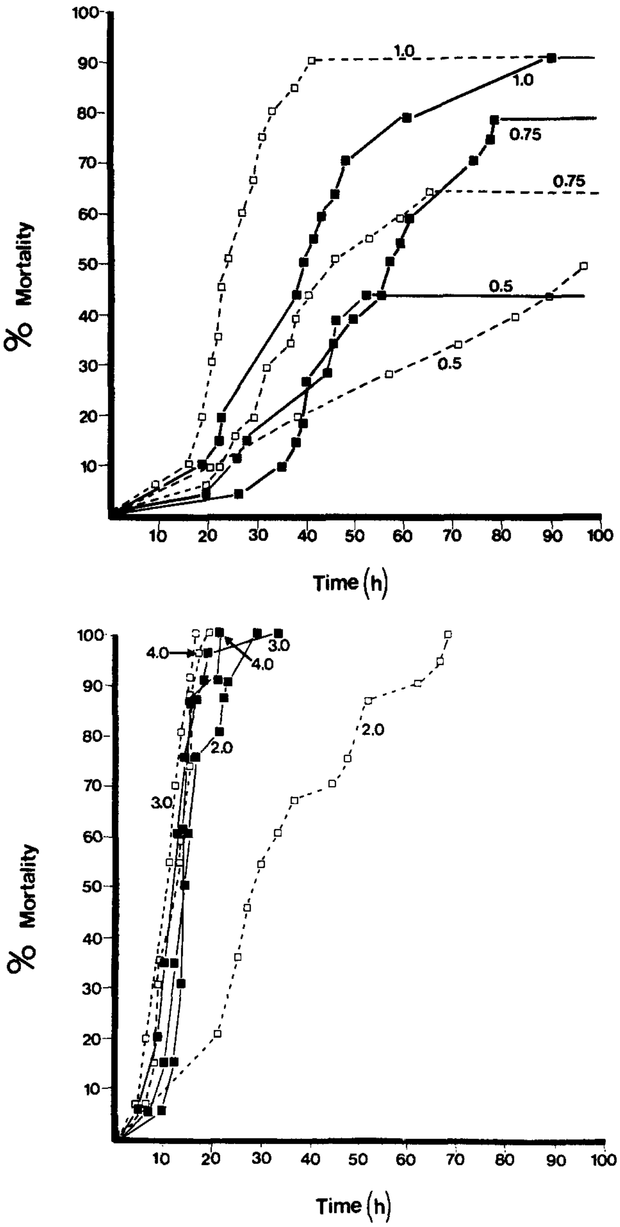

Fig. 4 A and B. Mortality curves at the different copper concentrations (mg/L) tested over time

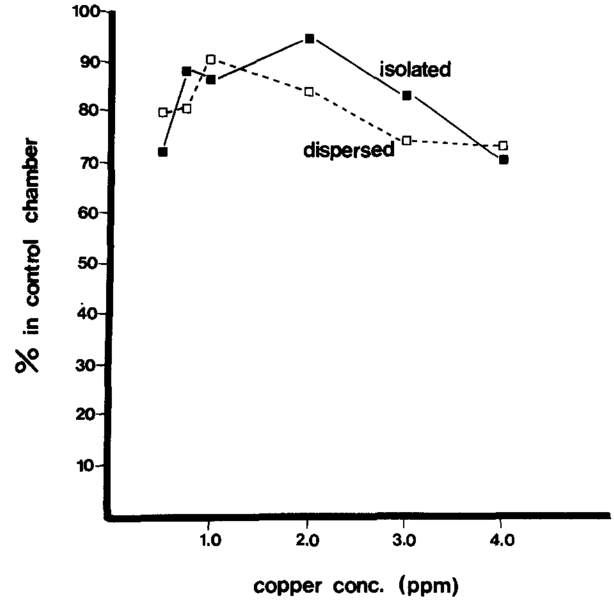

Fig. 5. Percentage of injured fish located in the less contaminated water, at the different copper concentrations (mg/L)

of a natural stream, confirming the findings of Giattina *et al.* (1982) and Black and Birge (1980) with sublethal levels.

At all test concentrations, reduced feeding was observed (normal rations were not consumed); this has been reported to be an indicator of stress (Lett *et al.* 1976). The vast majority of injured fish were recorded in less-contaminated water. The ability to recognize hazardous waters when injured seems to be highly developed. Higher levels of copper may render the sensory system dysfunction. Hara *et al.* (1976) proposed that copper binds to the olfactory receptors, interrupting sulfhydryl and amino groups of proteins from reacting with the receptor membranes, thus reducing the sensitivity of the olfactory bulb. The result is observed as stationary action by the fish which is perceived as attraction.

The different feeding techniques produced similar responses concerning attraction and avoidance stages over time (Figure 2). However, at lower levels, dispersed feeding exhibited an enhanced avoidance phase most prominent at the 1.0 mg/L level. At the higher concentrations, responses varied due to the lethality of the copper solutions. One significant conclusion is that increased levels of copper do not lead to enhanced avoidance, thus reducing mortality.

The results are in agreement with the findings of other work done with lethal levels (Black and Birge 1980) concerning preference and avoidance concentrations. We wish to stress that non-uniform contamination of water by copper may be as hazardous as uniform contamination if fish are attracted to the source of the contamination. Commonly found toxicants, such as copper, should be classified by their effect on behavior, with water quality standards adjusted accordingly. Furthermore, the location of dead and dying fish may not be in that area which has been contaminated, since our injured and dying individuals were virtually always found in less contaminated water. Such a phenomenon must be considered when examining environmental pertubations and clean up operations.

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References

Black, JA, Birge WJ (1980) An avoidance response bioassay for aquatic pollutants. University of Kentucky, Water Resources Research Institute, Research Report 123, Lexington, Kentucky

Brown VM, Shaw TL, Shurben DG (1974) Aspects of water quality and the toxicity of copper to rainbow trout. Water Res 8:797–803

Cripe C (1979) An automated device (AGARS) for studying avoidance of pollutant gradients by aquatic organisms. J Fish Res Board Can 36:11–16

Geckler JR, Horning WB, Neilheisel TM, Pickering OH, Robinson EL, Stephan CE (1976) Validity of laboratory tests for predicting copper toxicity in streams. United States Environmental Protection Agency. EPA-600/3-76-116, Environmental Research Laboratory, Duluth, Minnesota

Giattina JD, Gardon RR, Stevens DG (1982) Avoidance of copper and nickel by rainbow trout as monitored by a computer-based data acquisition system. Trans Amer Fish Soc 111:491–504

Gwillym RD (1984) Reclamation and pollution control at a severely disturbed minesite in northern New Brunswick, Canada. Minerals and the Environment 6:1–4

Hara TJ, Law YMC, McDonald S (1976) Effects of mercury and copper on the olfactory response in rainbow trout, *Salmo gairdneri*. J Fish Res Board Can 33:1568–1573

Ishio, S (1965) Behavior of fish exposed to toxic substances. International Conference on Water Pollut Res 2:19–40

Kleerekoper H (1976) Effects of sublethal concentrations of pollutants on the behaviour of fish. J Fish Res Board Can 33:2036–2039

Kleerekoper H, Westlake GF, Matis JH, Gensler PH (1972) Orientation of goldfish, *Carassius auratus* in response to a shallow gradient of a sublethal concentration of copper in an open field. J Fish Res Board Can 29:45–54

Leduc G (1966) Une bouteille à débit constant pour petits volumes de liquides. Le Naturaliste Canadien 93:61–64

Lett PF, Farmer GJ, Beamish FWH (1976) Effect of copper on some aspects of the bioenergetics of rainbow trout, *Salmo gairdneri*. J Fish Res Board Can 33:1335–1342

Spear PA, Anderson PD (1975) Fish size as a quantitative function of tolerance to heavy metals. Water Pollut Res Can 10:170–179

Spear PA, Pierce RC (1979) Copper in the aquatic environment: Chemistry, distribution and toxicology. National Research Council of Canada Publication No. 16454 Ottawa

Sprague JB (1964) Avoidance of copper-zinc solutions by young salmon in the laboratory. J Water Pollut Control Fed 36:990–1004

Timms AM, Kleerekoper H, Matis J (1972) Locomotor response of goldfish, channel catfish, and largemouth bass to a "copper-polluted" mass of water in an open field. Water Resour Res 8:1574–1580

Westlake GF, Kleerekoper H, Matis J (1974) The locomotor response of goldfish to a steep gradient of copper ions. Water Resour Res 10:103–105

Zar JH (1974) Biostatistical Analysis. Englewood Cliffs, NJ: Prentice-Hall

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