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THE BEHAVIOR OF THE AMPHIPOD *Gammarus lacustris* EXPOSED TO VARIOUS HYDROGEN ION AND COPPER CONCENTRATIONS IN A PREFERENCE-AVOIDANCE TROUGH

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ABSTRACT

de March, B.G.E. 1983. The behavior of the amphipod Gammarus lacustris exposed to various hydrogen ion and copper concentrations in a preference-avoidance trough. Can. Tech. Rep. Fish. Aquat. Sci. 1187: iv + 12 p.

Two experiments were conducted to demonstrate the effects of copper and of other chemical changes which accompany copper additions, on the behavior of the freshwater amphipod Gammarus lacustris. The first experiment examined the effects of only pH and distilled water; the second experiment, the effects of copper accompanied by unavoidable changes in pH and dilution with distilled water. Gammarus lacustris avoided reduced osmotic pressure and reduced pH, preferred increased pH, and moved more sporadically at increased pH values. In addition to the above responses, Gammarus lacustris avoided copper levels of 10^{-6} to 10^{-1} $mg{\cdot}L^{-1}$ $[Cu^{++}]$ calculated from equilibrium constants, preferred higher copper concentrations, and showed reduced locomotor activity at the lower copper levels. It was concluded that the avoidance reaction often observed at low total copper concentrations may result from associated lowered pH and possible reduced osmotic pressure. In addition, experimental design details relating to the toxicant-water interface may affect the response.

There were fewer interactions to explain when the results were presented in terms of pH and $[Cu^{++}]$, rather than in terms of pH and total dissolved copper.

Key words: chemical speciation; behavioral statistics; metal speciation; protective behavior; pH.

RESUME

de March, B.G.E. 1983. The behavior of the amphipod Gammarus lacustris exposed to various hydrogen ion and copper concentrations in a preference-avoidance trough. Can. Tech. Rep. Fish. Aquat. Sci. 1187: iv + 12 p.

Deux expériences ont été entreprises pour mettre en évidence les effets sur le comportement de l'amphipode d'eau douce Gammarus lacustris des variations dans la concentration du cuivre et d'autres produits chimiques qui accompagnent l'addition du cuivre. La première expérience ne s'est intéressée qu'aux effets du pH et de la dilution avec de l'eau distillée; la seconde a porté sur les effets du cuivre accompagné des inévitables changements de pH et de dilution. Gammarus lacustris a évité la pression osmotique réduite et le pH réduit; il a préféré le pH accru et se déplaçait plus sporadiquement dans le milieu ayant un pH accru. En plus des réactions indiquées ci-dessus, Gammarus lacustris a évité les concentrations de cuivre de 10^{-6} à 10^{-1} $mg{\cdot}L^{-1}$ $[Cu^{++}]$ calculées à partir des constantes d'équilibre; il a préféré les concentrations de cuivre plus élevées et il a ralenti son activité aux concentrations de cuivre les plus faibles. On a conclu que la réaction d'évitement souvent observée à de faibles concentrations de cuivre total était due à la réduction du pH et à la réduction possible de la pression osmotique. De plus, des détails de la conception expérimentale ayant trait à l'interface toxique-eau peuvent influer sur la réaction.

Il y avait moins d'interactions à expliquer lorsque les résultats ont été présentés en termes de pH et de $[Cu^{++}]$ plutôt qu'en termes de pH et de cuivre dissous total.

Mots-clés: spéciation chimique; statistiques du comportement; spéciation métallique; comportement de protection; pH.

INTRODUCTION

Knowledge of the ability of fish and invertebrates to sense and react to potentially dangerous toxicants is required for environmental decisions. Reported responses to heavy metals have been contradictory. The purpose of the present research is to explain some of these contradictions.

Jones (1947, 1948) was the first to report differing preference-avoidance responses at different concentrations of zinc, copper, and lead. Most of the studies since then report avoidance over a wide range of lethal and sublethal concentrations of copper (sometimes with zinc) (Sprague 1963, 1964; Sprague and Ramsay 1965; Grande 1967) and of zinc alone (Sprague and Ramsay 1965; Sprague 1968). Others have shown only preference, or preference and avoidance for similar test ranges (Ishio 1965; Kleerekoper et al. 1972; Westlake and Kleerekoper 1974; Maciorowski et al. 1977; Maciorowski et al. 1980). Another inconsistency is that Jones (1947) reported that animals showed reduced locomotor activity or became stupefied when preferring copper, whereas Hara (1981) and Kamchen and Hara (1980) reported that test animals maintained or increased their activity level (measured as number of interface crossing per unit time) when preferring copper.

The most accepted explanation of these discrepancies is that copper at different concentrations causes different responses, and that no other chemical or physical factors are involved (McLeese 1970; Hara 1981). Copper at lower concentrations causes sensory depression and is avoided, and at higher, causes sensory enhancement and is preferred. However, other factors may modify this response: extremely high copper concentrations may be avoided because of the blue water colour of the solution (Jones 1947); a steep copper gradient is avoided more strongly than a gentle one (Kleerekoper et al. 1972; Westlake and Kleerekoper 1974); and the response to changed temperature (Kleerekoper et al. 1973) or CO_2 (Costa 1967) can override the response to copper.

Although the addition of behaviorally detectable levels of copper to fresh water often causes changes in pH, hardness, alkalinity, CO_2, and osmotic pressure, these changes are seldom reported. In particular, the pH changes may be important since many organisms avoid water of low pH (Wells 1915; Jones 1948; Bishai 1962; Costa 1967; Laughlin et al. 1978; and Reynolds 1978). Also, the concentrations of particular copper species (eg. $[Cu^{++}]$, $[CuOH^{+}]$, and $[Cu(OH)_2]$), closely related to the above mentioned chemical factors, may be the actual causes of responses, as has been shown for acute mortality (Pagenkopf et al. 1974; Andrew et al. 1977; Howarth and Sprague 1978; Wagemann and Barica 1979; Dodge and Theis 1979; Miller and MacKay 1980).

In preliminary studies, strong and often contradictory differences in the response of Gammarus lacustris to the same copper concentrations were noted. These differences could be attributed to the pH or amount of stock solution, and (or) to the timing of the introduction of the copper stock solution into the background water. Therefore, the described experiment studying the reaction of Gammarus lacustris to simultaneous changes in hydrogen and copper ion concentrations was conducted. Any other possibly relevant chemical factors such as the concentration of the various copper species could be calculated from these variables and the known water chemistry (Table 1).

MATERIALS AND METHODS

All tests were conducted with adult-sized, unpaired Gammarus lacustris (Crustacea:Amphipoda:Gammaridae) from Lakes 101 and 255 (Sunde and Barica 1975) near Erickson, Manitoba (50°30'N, 100°10'W). Animals were collected in the fall, and were held for up to six months at 16 hour daylengths which prevented the sexual maturation of the females (de March 1982). Animals were held at 15°C in tanks with a moderately heavy algal growth and with a water replacement rate of 50%/week. They were fed to satiation with Tetra-Min B (Tetra-Werke, West Germany) three times a week, but not on the day of testing since G. lacustris will not move readily if recently fed.

The apparatus (Fig. 1) was a modification of the one described by Maciorowski et al. (1977). Each long end of the chamber was constructed as two separating pieces, with 450 μm netting separating the pieces. This netting was less attractive to animals than end baffles with large and irregular holes. Animals still often preferred one end in their pretest behavior, but not all chose the same one. In this chamber, water came from one vessel, and was separated into two streams just before it entered the test trough.

The inner dimensions of the test trough, (Fig. 1) excluding the mixing areas outside the netting, were 4 cm wide x 21 cm long x 9 cm high, with water depth usually 2.5 cm. The stock solution containing either copper, and (or) with a modified pH, was introduced into one of the two mixing chambers and was mixed into the water by vigorous aeration. This prevented time-related precipitation of copper from affecting the copper concentrations.

Animals were tested in u.v. dechlorinated tap water (Table 1) entering the trough at 62.1 $mL \cdot min^{-1}$ (or 6.21 $mL \cdot min^{-1} \cdot cm^{-2}$) at each end. Although this water often contained up to 10 $\mu g \cdot L^{-1}$ of copper, previous experiments suggested that the copper was in an inactive form, since the addition of even a very small additional amount of dissolved copper (as low as 1 $\mu g \cdot L^{-1}$) elicited a behavioral response. The stock solution was dripped into one end of the chamber at 3.08 $mL \cdot min^{-1}$, a 5% introduction rate, the lowest that could be maintained with the available pumps. In each animal's test the pH was measured in both ends of the chamber four minutes after the toxicant was introduced into one end. Copper concentrations were confirmed by flame atomic absorption spectroscopy once or twice at every experimental concentration, and dilution rates were confirmed once a day.

The predicted concentrations of various species of copper in the test water were obtained from a computer program designed by Wagemann et al. (in prep). The program predicts the concentration of 12 soluble copper species. However, since the concentrations of several of these were highly correlated with each other, only $[Cu^{++}]$, $[CuOH^{+}]$, and $[Cu(OH)_2]$ were considered in the initial data analysis. After running the program, I obtained my own equations from the output which related the fraction of copper present as a particular species to the pH of the test water (Table 2) so that species concentration could be calculated directly, rather than by interpolation from a table. $[Cu^{++}]$ was dominant at the lower pH values; $[CuOH^{+}]$, at the intermediate pH values, and $[Cu(OH)_2]$, at high pH values. In this paper, [Sp] will refer to the calculated concentration of a species.

The movements of animals were recorded on a strip chart by the method described by Scherer and Nowak (1973) (Fig. 1). Each animal was tested for 10-20 passes (trips) into each end of the trough before the toxicant was introduced (pretest run). Then each animal was tested again after the introduction of the stock solution (test run). In each run, the recording of the movement commenced when the animal entered the left end, and terminated when it left the right end 20 trips or 7 minutes later. The end at which the toxicant was introduced, the "toxicant" end as opposed to the "clean" end, was randomized and recorded.

Preliminary tests showed that G. lacustris avoided water containing 5% distilled water, or containing 5% distilled water adjusted to any pH with H_2SO_4 or NaOH. These solutions had conductivities up to 10 $\mu S \cdot cm^{-1}$. Animals did not avoid distilled water at pH 7 to 9 adjusted to 200 $\mu S \cdot cm^{-1}$ with NaOH and H_2SO_4. It was thus believed that distilled water was avoided because of its osmotic properties. Since both the pH and the conductivity of the copper stock solutions could not be adjusted without forming precipitates, the avoidance of distilled water was considered to be a complication which could not be eliminated.

Two experiments were performed: the first to determine how animals responded to the introduction of various hydrogen ion concentrations, and the second, to determine how animals responded to the simultaneous introduction of hydrogen and copper ions. By comparing the results of the two experiments the effects of copper alone could be identified.

pH EXPERIMENT

An average of eight animals was tested in each of six experimental categories, these being (3 ranges of background water pH) x (upward or downward change in pH). The allocation of test values is shown in Table 4.

The background water was adjusted to one of 3 pH ranges (pH 4-7, 7-8, and 8-9) with H_2SO_4 or NaOH. The stock solution, dripped into the background water during the test run, consisted of distilled water adjusted to various pH values with H_2SO_4 or NaOH.

More animals were tested with background water of pH 7.8 to define more closely the minimum pH change causing behavioral changes. Animals were not acclimated to the pH of the background water.

(COPPER AND pH) EXPERIMENT

In this experiment, only background water of pH 7.8 was used. The stock solution dripped into one end of the test chamber during the test run was adjusted to both a desired pH and copper concentration. The final allocation of test values is shown in Table 4. Final test concentrations ranged from $5x10^{-5}$ to $5x10^{0}$ $mg \cdot L^{-}$ dissolved copper, and from pH 4.0 to 9.3. An average of ten animals was tested at each concentration. More animals were tested at $5x10^{-2}$ and $5x10^{-1}$ $mg \cdot L^{-1}$ since both preference and avoidance responses were evident at these copper concentration.

STATISTICAL METHODS

The "% time in an end" statistic, traditionally used for similar experiments (Sprague 1964), was not used here for reasons discussed by de March and Scherer (1980). That paper makes alternative suggestions for the analysis of such data, and these suggestions are used in this study as follows. Since the movement of each animal was recorded as a wave-like strip chart tracing, (Fig. 1) the component "trip times" (i.e. the time elapsed between entering an end and leaving it) for each end of the trough could be measured for the control and toxicant runs. The individual trip times tested were log-transformed. This transformation satisfied Barlett's test of homogeneity of variances for control run trip times (Snedecor and Cochran 1967). The mean log trip times (MLTT's) for each animal and their standard deviations (SD's) were calculated for each of four categories: both the toxicant and clean ends during both the pretest and test runs. In the pH Experiment the SD's of the MLTT's increased during the test run. Thus SD was treated as another dependent variable in the statistical analyses for this experiment.

The MLTT's and one mentioned SD in both ends from the toxicant run were treated as the dependent variables in multiple regression analysis, while the same measurements from the pretest run were treated as covariates or independent variables. Additional independent variables were: the end at which the toxicant was introduced (left = -1; right = +1), pH in both ends during the test run, and total dissolved copper or $[Cu^{++}]$, both in log $(mg \cdot L^{-1})$. Squares and cross products of independent variables were treated as additional independent variables. The independent variables were used to predict the dependent using the MAXR forward stepwise selection procedure described in SAS (SAS Institute Inc. 1979). This method finds the best 1 variable model, 2 variable model,... n variable model by sequentially attempting all possible switches in equations. The most inclusive models with all variables significant at the α = 0.01 level were chosen.

Pairs of pretest and test run equations from the regressions were subtracted or added to tive predictive expressions for choice (preference or avoidance) or for activity level (number of trips per minute) respectively. These expected values are plotted on all figures. These sums or differences can be converted to estimates of traditional indices if desired. If D = the difference between two MLTT's then % time in one end can be estimated by $(10^D)/(1 + 10^D) \times 100$. If S = the sum of two MLTT's, then the number of trips per minute can be estimated by $60/10^{(S/2)}$.

RESULTS

The soft water used for these experiments had a weak buffering capacity. Stock solutions of pH 2, 4, and 6 introduced at a 5% dilution rate into water at pH 7.8 gave mixtures near pH 7.35, 7.55 and 7.7 respectively.

Animals placed into the trough moved back and forth from end to end (netting to netting) regularly, spending an average of 10 seconds (MLTT = 1.0) in each end. They did not appear to experience any shock or trauma when transferred to the test system, since they commenced swimming immediately, and slowed down only slightly within 1/2 hour.

Table 3 shows the final equations for predicting all MLTT's and one SD. Overall, the order of incorporation of variables and the final partial F-values (both not shown) showed that responses were equally strongly related to 1) the control run MLTT's; 2) pH values in both ends of the chamber (when applicable), and 3) copper concentrations when applicable. For this reason, plots or tabulations of responses in terms of only one or two independent variables nearly always show neglible trends or differences. Two simple responses, the difference between and the sum of the MLTT's in both ends during the test run in various ranges of pH and copper values, shown in Table 4, show the main trends. However, if one uses the MSE's from the final models (Table 3), and the correlations between the estimated responses (not shown), standard deviations on this table would be reduced by about 2/3. Because of the multivariate nature of these responses, the data should be viewed in terms of the final models rather than the means in Table 4.

The expression for expected choice (preference or avoidance) in the pH experiment was obtained from Equations [1] - [2]; an expression for activity level, from equations [1] + [2] (Table 3). Figure 2a shows the response surfaces for the expected choices, and Fig. 2b, for expected activity levels for animals with control MLTT's = 1.0. Animals generally avoided the introduced solution, with the degree of avoidance proportional to the pH decrease. Solutions with no pH change were avoided slightly. An increase of 1 pH unit generally elicited no response, while larger increases were preferred. The no-choice isolines for animals with slighty slower or faster control MLTT's are shown with dotted lines. Slower animals with a control trip time of 15 seconds (retransformed from MLTT = 1.18) avoided reduced pH more strongly than those with MLTT = 1.0. Faster animals with a control trip time of 7.0 seconds (MLTT = 0.85) avoided reduced pH less strongly.

Activity levels in this experiment did not change when the pH at either end of the trough was in middle ranges (Fig. 2b). Strong preference or avoidance reactions (Fig. 2a) were usually accompanied by decreases in activity. Animals slowed down considerably when pH changes occurred only in high or only in low range. Animals which were slower in the pretest runs slowed down less, and faster ones, more.

The standard deviation of the MLTT in the toxicant end changed with pH in this experiment (Equation [7], Table 3). The mean SD during the control runs was 0.313 MLTT units, and during the test run, 0.351 MLTT units. The equation suggests that the SD change was directly proportional to the pH increase. The SD increase was slightly larger when the toxicant was introduced on the right side, in this case, the side where the animal was when the toxicant was introduced.

Expressions for choice and activity levels in the (Copper and pH) experiment were obtained similarly, that is, choice was expressed by combining Equations [3] - [4], and activity levels as Equations [3] + [4], or the analogous equations [5] and [6]. Figure 3a shows the no-choice isoline, obtained from the pH experiment, for the tested ranges for animals with control MLTT = 1.0 for various final pH values. Even though avoidance was observed in most of the experiment, the degree of avoidance was less than expected from pH changes alone. Preference was observed only at the two highest copper concentrations. In general, the tendency to avoid lowered pH seemed to be offset by copper, with a stronger copper concentration having a stronger effect. The lowest copper concentrations were preferred more than expected on the basis of pH changes at the high pH values (Fig. 3a).

Any final predictions containing both copper and pH variables can be algebraically manipulated to give predictions in terms of pH and any copper species using the equations in Table 2. Such equations would have a predictive capacity identical to the original ones, but the relative importance of various terms would change. Thus a choice between models can be made only on the basis of subjective criteria such as linearity preferred to curvilinearity, or addivity of factors preferred to interactivity. The only copper species which did not yield models which were conceptually more complex than the model with total dissolved copper was $[Cu^{++}]$.

The equivalent statistic and graph for results in terms of $[Cu^{++}]$ from Equations [5] and [6], Table 3, suggest a simpler explanation (Fig. 3b). It suggests that the effects of pH change and $[Cu^{++}]$ may have been additive, with concentrations of $[Cu^{++}]$ above 5×10^{-4} $mg \cdot L^{-2}$ $[Cu^{++}]$ preferred, and lower levels avoided. The effects of pH were as in the previous experiment.

Figure 3c shows the expected activity level isolines in the tested ranges at various final pH values. Activity levels and choice appear to be correlated. A high degree of preference of copper was generally accompanied by small activity level changes. Avoidance of copper was accompanied by considerable slowing. The parallel plot in terms of [Cu^{++}] (Fig. 3d) suggests that the slowing effects of pH and [Cu^{++}] are not completely independent, although the explanations may be simpler than that required for Fig. 3c. Animals moved faster only when both pH and copper concentrations were high.

In general, slower animals avoided the toxicant end more strongly, and also slowed down more in response to the toxicant mixture (coefficients of pretest MLTT's, Equations [3] to [6], Table 3).

DISCUSSION

The results of these experiments suggest that a high percentage of the avoidance of copper observed in preference-avoidance experiments may be due to lowered pH, and possibly, reduced osmotic pressure. The pH changes observed in the (Copper and pH) experiment most likely were similar to those in experiments described in the literature, thus their effect may have been similar. Nearly all heavy metal stock solutions, including copper, made according to laboratory standards, are acidified to pH 1 to 3 (Price 1972) if their pH is not already low. Such solutions must acidify fresh water unless it is of exceptionally high alkalinity. Unfortunately, the nature of the stock solutions and pH changes seldom are reported. Avoidance most likely would be recorded at low copper concentrations if stock solutions were highly acidified.

Low concentrations of copper, between 10^{-6} to 10^{-11} $mg \cdot L^{-1}$ [Cu^{++}] are, however, avoided more strongly than expected on the basis of pH change alone. The overall conclusion must still be that copper causes two responses, thus supporting the physiological observations made by Hara (1981). The higher copper range causes sensory enhancement, and the lower, sensory depression. Animals appear to slow down considerable at these low concentrations, even though they avoid them.

Preference and avoidance experiments such as these may not be an efficient method for elucidating multiple effects of one causal factor, or the interactive effects of several factors. The responses of choice, changes in activity levels, and erraticism are inseparably related. A "control experiment" examining the effects of pH and distilled water was done; perhaps another to examine activity levels in a situation where animals had no choice would have been appropriate. Also, current direction may have been a confounding factor. Each animal was given two choices in the toxicant end: avoiding the toxicant by swimming with the current, or preferring it by swimming into the current. Organisms may have evolved to swim into an undesirable medium to get through it as fast as possible, or to swim with the current to maximize the contact time.

Experimental design differs greatly among preference-avoidance experiments reported in the literature. All authors who reported only avoidance reactions to copper (eg. Sprague 1963, 1964; Sprague and Ramsay 1965) tested each animal at a series of concentrations, from low to high, thus confounding test concentration with time, possible learned or cumulative responses, and acclimation. It is possible, given our results, that in some of these tests, animals recognized and avoided the dilute water or reduced pH, and then maintained and enhanced this behavior pattern. Authors who reported both preference and avoidance did not use such an experimental designs.

The gradient of the toxicant interface encountered may also have affected the results. Slower animals generally showed an expected response more strongly than faster animals (Coefficients of MLTT's, Table 3). It may be that slower animals encountered a relatively well-established interface at an early pass into the toxicant end, and thus responded more strongly at an earlier trip. Westlake and Kleerekoper's (1974) direct demonstration of different responses to shallow and steep gradients is supported by our observations. Thus, the variability in the data might have been reduced if animals had been forced to stay in the clean end until the interface was fully established.

Regression statistics are a powerful tool for analyzing data in which responses are caused by one or more of a number of either orthogonal or random factors. The practical limits of the test system make it impossible to hold all but one of the desired factors constant. However, the strength of the technique is lessened when a number of intercorrelated independent variables such as pH and the copper species are used in analyses. Although it is possible that [Cu^{++}] was the causal factor in our experiments, the experiment was not set up to distinguish between the importance of the various species, so the interpretation is still subjective due to experimental confounding.

These findings certainly confirm that this type of behavioral test is an extremely sensitive indicator of changes in water chemistry. The difficulty with its use in monitoring or classifying toxicants would be one of ensuring that one is really testing the variable that is being studied. The extrapolation of such laboratory data to field conditions could be tenuous.

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Table 1. Physical and chemical features of Freshwater Institute water.

Temperature	15°C	
Conductivity	200	$\mu S \cdot cm^{-1}$
K^+	1.16	$mg \cdot L^{-1}$
Na^+	1.72	$mg \cdot L^{-1}$
SO_4	7.7	$mg \cdot L^{-1}$
Mg^{++}	5.84	$mg \cdot L^{-1}$
Ca^{++}	22.87	$mg \cdot L^{-1}$
Cl^-	6.00	$mg \cdot L^{-1}$
NH^3-N	.030	$mg \cdot L^{-1}$
Total dissolved inorganic carbon	720	$\mu mole \cdot L^{-1}$
Dissolved organic carbon	900	$\mu mole \cdot L^{-1}$
pH	7.8	
Hardness (as $CaCO_3$)	80	$mg \cdot L^{-1}$

Table 2. Equations for predicting chemical speciation in Freshwater Institute u.v. dechlorinated water between pH = 4.0 and pH = 9.0 calculated from output of the computer program by Wagemann et al. (1980). $R^2 >$ 0.998 for all equations. n = 51.

Log fraction $[Cu^{++}] = 2.62\ pH - 0.246\ (pH)^2 - 6.447$.

Log fraction $[CuOH^+] = -3.63\ pH - 313.84/pH + 493.51\ (pH*pH) + 58.71$.

Log fraction $[Cu(OH)_2] = -46.09/pH + 5.57$.

Therefore,

log [Species concentration] = log [total copper added] + log fraction [species].

Table 3. Equations relating Mean log trip times (MLTT's) and one standard deviation (SD) of the MLTT's in the clean and toxicant ends in terms of all possible independent variables, including corresponding control run MLTT's. The coefficients for both control run MLTT's were combined. T = Control run MLTT, B = pH clean end, A = pH toxicant end, S = end of toxicant introduction (L =-1, R = 1), U = total dissolved copper concentration in log ($mg \cdot L^{-1}$), C = calculated [Cu^{++}] concentration in log ($mg \cdot L^{-1}$).

Dependent MLTT	Equation	R^2	df	F	MSE
<u>pH Experiment</u>					
[1] Tox End	$= -.0678B + 0698A + 0.933.$	0.359	2,48	13.42	0.0280
[2] Clean End	$= 1.74B - .142\ AT + 0.0114\ AB -0.217.$	0.436	3,47	12.10	0.0290
<u>(Copper and pH) Experiment</u>					
[3] Clean End	$= 0.0630A + 0.555T^2 - 0.104UT +0.0232\ U^2 + 0.0199.$	0.331	4,60	7.42	0.0388
[4] Tox End	$= 0.456U + 0.335T^2 - 0.0488\ AT -0.216UT - 0.0423AU + 0.968.$	0.485	5,59	11.11	0.0250
<u>(Copper and pH) Experiment</u>					
[5] Clean End	$= 0.464A + 0.700T^2 + 0.116CT -0.0309A^2 + 0.0132C^2 - 1.22.$	0.400	5,59	7.86	0.0354
[6] Tox End	$= 0.324C - 0.207CT - 0.0254CB -0.00805A^2 + 1.25.$	0.510	5,59	12.26	0.0233
<u>pH Experiment</u>					
[7]	$SD=-.240S - .0684BT + .0568AT +0.275\ ST - .240.$	0.392	5,45	5.81	0.0103

Table 4. Mean MLTT's ± 1 SD in the toxicant and clean ends during the toxicant run in various test categories in the two experiments. ΔMLTT is the difference between the two MLTT's; Σ MLTT, the sum of the two MLTT's; n, the number of animals tested in a category.

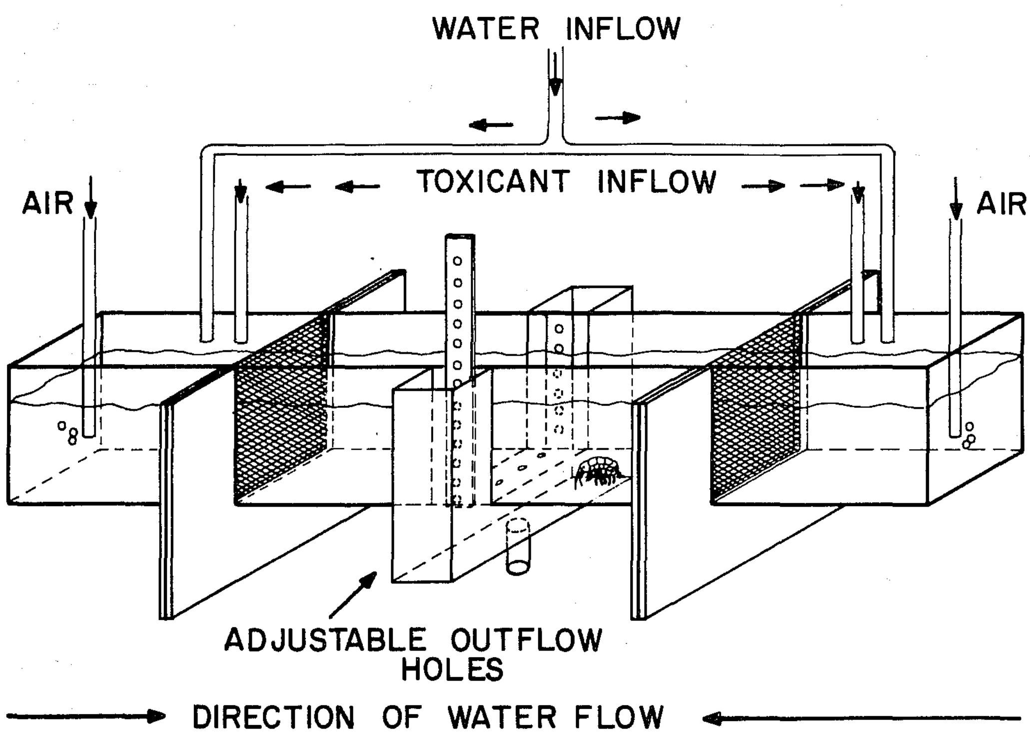
pH Experiment

pH Background Water	pH Change (pH units)	MLTT Toxicant End	MLTT Clean End	Δ MLTT's	Σ MLTT's	n
4-7	0.0 to 2.30	1.00 ± 0.189	1.03 ± 0.220	-0.026	2.03	13
	-0.03 to -0.20	0.854± 0.335	1.014± 0.335	-0.160	1.87	5
7-8	0.15 to 1.60	1.05 ± 0.145	1.04 ± 0.253	0.00500	2.09	8
	-0.05 to -0.80	0.909± 0.240	1.18 ± 0.175	-0.266	2.08	18
8-9	0.05 to 0.90	1.09 ± 0.183	1.17 ± 0.189	-0.078	2.26	5
	-0.45 to -0.50	0.810± 0.0707	1.10 ± .134	-0.295	1.92	2

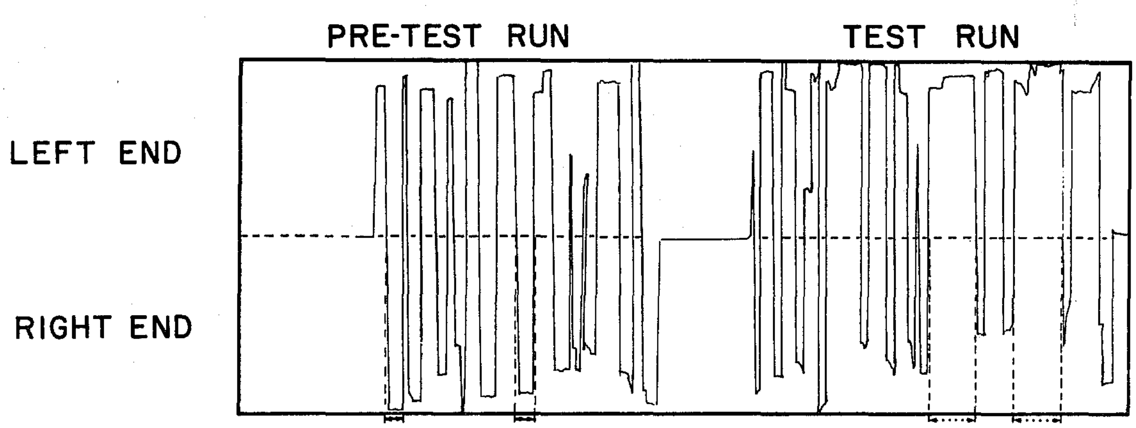
(Copper + pH) Experiment

Final Copper Concentration ($mg \cdot L^{-1}$)	pH Range	MLTT Toxicant End	MLTT Clean End	Δ MLTT's	Σ MLTT's	n
5×10^{-5} and 5×10^{-4}	6.20 to 7.40	1.01 ± 0.183	1.17 ± 0.193	-0.161	2.18	11
	7.55 to 8.80	1.00 ± 0.113	1.11 ± 0.187	-0.0860	2.11	8
5×10^{-3} and 5×10^{-2}	6.80 to 7.40	0.754 ± 0.236	1.14 ± 0.225	-0.318	1.89	7
	7.60 to 8.80	0.916 ± 0.201	1.037 ± 0.206	-0.121	1.95	17
5×10^{-1} and 5×10^{0}	4.00 to 7.40	1.00 ± 0.322	0.993 ± 0.150	0.00882	2.00	17
	7.80 to 9.30	0.940 ± 0.0728	0.796 ± 0.238	0.144	1.74	5

PREFERENCE - AVOIDANCE TROUGH



STRIP CHART RECORDING



←——→ REPRESENTS VARIOUS MEASURED TRIP TIMES IN THE RIGHT END

←········→ REPRESENTS VARIOUS MEASURED TRIP TIMES IN THE LEFT END

Fig. 1. The preference-avoidance trough and a strip-chart recording from one animal's performance. Drawings are not to scale.

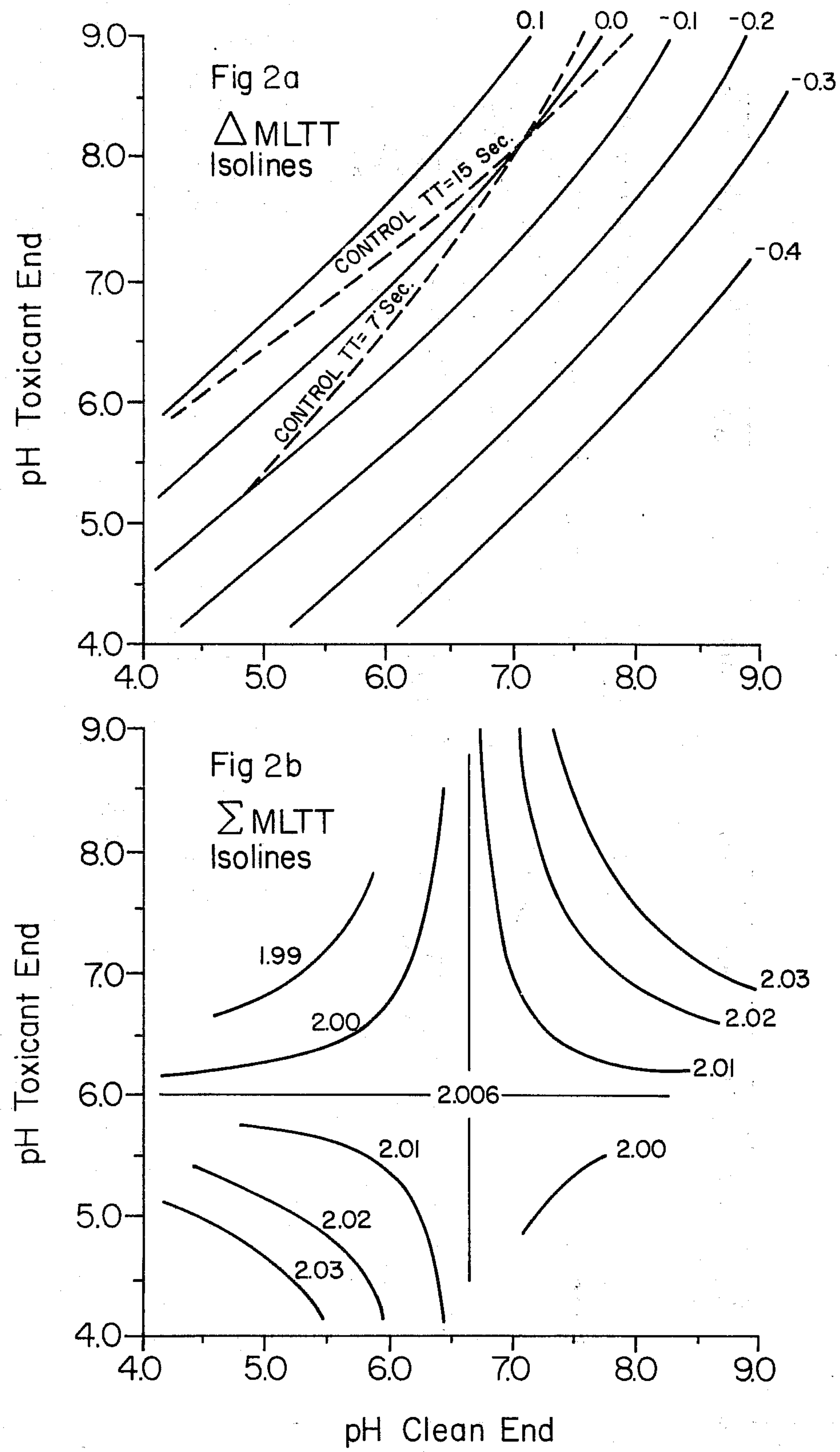


Fig. 2. Predicted response curves from Equations [1] and [2], Table 3, for animals with control MLTT's = 1.00 (unless otherwise marked) in the pH experiment. ΔMLTT, indicating choice, is the predicted (MLTT toxicant end - MLTT clean end) during the test run. A negative ΔMLTT indicates avoidance. Σ MLTT, indicating activity, is the sum of the two MLTT's. Σ MLTT = 2.0 indicates no change in activity level.

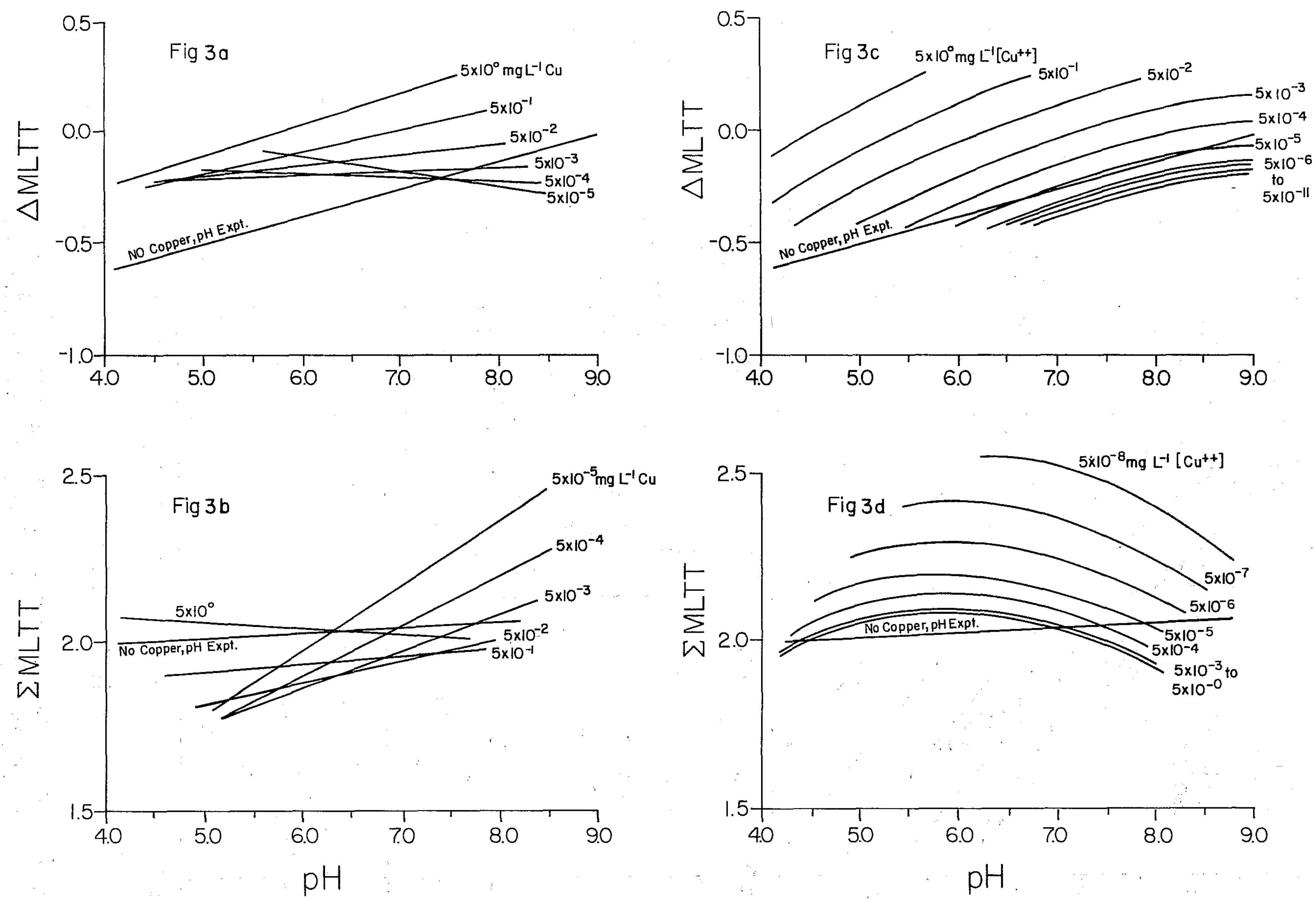


Fig. 3. Predicted response curves from Equations [3] and [4], and [5] and [6] (Table 3) for animals with control MLTT's = 1.00 in the pH and copper experiment. ΔMLTT and Σ MLTT have same meanings as in Figure 2. Cu refers to total dissolved copper, $[Cu^{++}]$ to the concentration of this species calculated from equilibrium constants.