# BEHAVIORAL AVOIDANCE BY FISH OF RESIDUAL CHLORINE IN POWER PLANT DISCHARGES

BY

Stephan Richard Larrick

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APPROVED:

Kenneth L. Dickson, Chairman

Donald S. Cherry

Alan G. Heath

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# I. INTRODUCTION

The number of steam electric power plants that are proposed, under construction, or in full operation has dramatically increased in recent years. It has been estimated that electric power demand has been doubling in the United States every six to ten years (Cairns, 1972). Thus, the potential for environmental impact is great. To increase the plants' turbine efficiency, a partial vacuum is created by cooling and condensing the steam exhaust leaving the turbine, passing it to water cooled condensers (Alabaster, 1963). Biocides, such as chlorine, are commonly introduced into the condenser tubes to prevent the growth of organisms which insulate the tubes (Mattice and Zittel, 1976) or create excessive back-pressure (Beauchamp, 1969) both of which reduce the plants' operating efficiency. Other chemicals are added to prevent corrosion, to clean certain surfaces or for a variety of other purposes (Becker and Thatcher, 1973). When the cooling water is returned to the river or lake, non-target organisms are exposed to these chemicals and the elevated temperatures.

The environmental impact of power plant discharges on non-target organisms is difficult to assess. Some studies have shown that planktonic communities (Markowski, 1959), fish communities (Markowski, 1962) and the hatching success of fish eggs (Schübel, 1974) are not affected adversely by cooling water discharged by power plants. Generally, many fish species are actually attracted to the warmer water (Allen *et al*., 1970; Barans and Tubb, 1973;

Marcy *et al.*, 1972; Stauffer *et al.*, 1974). Other authors have found that some larval fish are adversely affected by power plant discharges (Marcy, 1971).

Chlorine is an important constituent of many power plant discharges and its toxic effects are well documented (Doudoroff and Katz, 1954; Brungs, 1973; Mattice and Zittel, 1976). Unfortunately, most early studies measured only total residual chlorine (TRC) and ignored the combined (CRC) and free residual chlorine (FRC), e.g. Panikkar (1960). Other studies focused on combined residual chlorine, which predominates in domestic wastewater (Merkens, 1958; Zillich, 1972). More information is needed concerning free residual chlorine and the possible synergistic effects of pH and temperature on chlorine toxicity. The above static bioassays assume that aquatic organisms remain stationary in the toxic solution: a premise easily accepted for sessile organisms. However, if the discharge canal is more than a trivial part of the total available habitat, the avoidance of the plume may be an important element of ecological plasticity for motile organisms such as fish (Coutant, 1975). Field studies have demonstrated that fish do indeed avoid polluted waters (Katz and Gaufin, 1952; Sprague *et al.*, 1965; Tsai, 1969,1970).

The present study examines four areas of chlorine avoidance behavior by fish that have, as yet, not been investigated. The study objectives are: (1) to determine if avoidance behavior is correlated with a specific component of the total residual chlorine (combined residual chlorine, free residual chlorine, or the hypochlorous acid concentration) (2) to evaluate the influence of acclimation temperatures on the avoidance behavior of fish (3) to investigate the possible modifications in avoidance behavior by steep thermal gradients and (4) to determine the concentration of chlorine avoided (threshold) by fish and ascertain whether this level is lethal or sublethal.

## II. LITERATURE REVIEW OF AVOIDANCE STUDIES

Several techniques are currently available for use in behavioral avoidance studies (TABLE 1). Each was designed for a specific application and prospective investigators must select the most appropriate technique for their problem. To facilitate this selection process, the merits and shortcomings of previous behavioral studies will be evaluated.

The various experimental troughs used in avoidance studies may be divided into two categories -- troughs that produce shallow gradients and others that produce steep gradients. Shelford and Allee (1913), the original proponents of fish behavioral studies as related to water quality, felt that it was impossible to study the reaction of fishes to dissolved gases without the use of shallow concentration gradients. Their apparatus consisted of two parallel boxes, each 120 cm long and enclosed by curtains to minimize external disturbances. Untreated water entered both ends of the reference box. In the experimental box, untreated water entered at one end while modified water was introduced into the opposite end, creating three relatively distinct regions: a region at each end filled with the water introduced on that side, and a central mixing zone. An experimental trial consisted of placing one fish in each trough and recording the fish's movements on graph paper. Horizontal tracings represented the relative position of the fish in the trough and vertical distance represented residency time. Equal residency times and/or equal number of entrances into each side of the trough indicated a neutral response to a toxicant. This "gradient method" was

TABLE 1 - A Review of Fish Avoidance Studies.

| Reference | Species | Chemical |
|---|---|---|
| | Methods Creating Shallow Toxic Gradients | |
| Shelford and Allee, 1913. | Abramis crysoleucas (Mit.) | $CO_2$ |
| | Amboplites rupestris (Raf.) | |
| | Ameiurus melas (Raf.) | |
| | Catostomus commersonii (Lac.) | |
| | Etheostoma coeruleum (Stor.) | |
| | Hybopsis kentuckiensis (Raf.) | |
| | Lepomis cyanellus (Raf.) | |
| | Micropterus dolomieu (Lac.) | |
| | Notropis cornutus (Mit.) | |
| | Umbra limi (Kirt.) | |
| | Abramis crysoleucas (Mit.) | $O_2$ |
| | Amboplites rupestris (Raf.) | |
| | Catostomus commersonii (Lac.) | |
| | Hybopsis kentuckiensis (Raf.) | |
| | Lepomis cyanellus (Raf.) | |
| | Notropis cornutus (Mit.) | |
| | Hybopsis kentuckiensis (Raf.) | $N_2$ |
| | Notropis cornutus (Mit.) | |
| | Abramis crysoleucas (Mit.) | Boiled $H_2O$ |
| | Amboplites rupestris (Raf.) | |
| | Ameiurus melas (Raf.) | |
| | Catostomus commersonii (Lac.) | |
| | Etheostoma coeruleum (Stor.) | |
| | Hybopsis kentuckiensis (Raf.) | |
| | Lepomis cyanellus (Raf.) | |
| | Micropterus dolomieu (Lac.) | |
| | Notropis atherinoides (Raf.) | |
| | Notropis cornutus (Mit.) | |
| | Abramis crysoleucas (Mit.) | Boiled $H_2O$ + $O_2$ |
| | Amboplites rupestris (Raf.) | |
| | Catostomus commersonii (Lac.) | |
| | Hybopsis kentuckiensis (Raf.) | |
| | Lepomis cyanellus (Raf.) | |
| | Micropterus dolomieu (Lac.) | |
| | Notropis cornutus (Mit.) | |

TABLE 1 - (Continued).

| Reference | Species | Chemical |
|---|---|---|
| | Shallow Gradients (cont.) | |
| Shelford and Allee, 1913. | Abramis crysoleucas (Mit.)<br>Amblopites rupestris (Raf.)<br>Ameiurus melas (Raf.)<br>Catostomus commersonii (Lac.)<br>Hybopsis kentuckiensis (Raf.)<br>Lepomis cyanellus (Raf.)<br>Notropis cornutus (Mit.)<br>Umbra limi (Kirt.) | Boiled $H_2O$ + $CO_2$ |
| Shelford and Allee, 1914. | Abramis crysoleucas<br>Amboplites kentuckiensis<br>Ameiurus melas<br>Catostomus commersonii<br>Etheostoma zonale<br>Hybopsis kentuckiensis<br>Lepomis cyanellus<br>Micropterus dolomieu<br>Notropis cornutus<br>Umbra limi | $O_2$, $CO_2$,<br>Boiled $H_2O$<br>Boiled $H_2O$ + $CO_2$ |
| Wells, 1915. | Ameiurus melas<br>Lepomis pallidus<br>Pomoxis annularis | $H_2CO_3$ |
| | Ameiurus melas<br>Lepomis pallidus | $H_2SO_4$ |
| | Ameiurus melas<br>Lepomis pallidus<br>Pomoxis annularis | $Na_2CO_3$ |
| | Lepomis pallidus | $NH_4OH$ |
| Ishio, 1964. | Acheilognathus limbata<br>Carassius auratus<br>Cyprinus carpio<br>Moroco steindachneri<br>Tribolodan hakonensis | pH |

TABLE 1 - (Continued).

| Reference | Species | Chemical |
|---|---|---|
| | Shallow Gradients (cont.) | |
| Ishio, 1964. | Acheilognathus limbata<br>Carassius auratus<br>Cyprinus carpio<br>Gnathopogon gracilis<br>Lebistes reticulatus<br>Lepomis machrochirus<br>Moroco steindachneri<br>Pimephales promelas<br>Pungtungia herzi<br>Sarchocheilichthys variegatus<br>Zacco platypus | $OH^-$<br>$H_2CO_3$<br>$CuCl_2$<br>$NH_4OH$ |
| Summerfelt and Lewis, 1967. | Lepomis cyanella<br>Lepomis megalotes<br>Lepomis microlophus<br>Notemigonus crysoleucas | a-Chloro-acetophenone (ACP) |
| | Lepomis cyanella | 1-4-Dichloro-2-Nitro-benzene |
| Stott and Cross, 1973. | Rutilus rutilus L. | $O_2$, $CO_2$ |
| | Methods Creating Steep Toxic Gradients | |
| Jones, 1947. | Pygosteus pungitius L. | Alcohol<br>Chloroform<br>Formalin<br>Mercuric-chloride<br>Zinc sulphate<br>Copper - sulphate |

TABLE 1 - (Continued).

| Reference | Species | Chemical |
|---|---|---|
| | Steep Gradients (cont.) | |
| Jones, 1948. | Gasterosteus aculeatus | Sodium-sulphide<br>Lead nitrate<br>Calcium nitrate<br>pH |
| | Phoxinus phoxinus | Lead nitrate<br>Zinc sulphate |
| Jones, 1951. | Phoxinus phoxinus | Phenol<br>Para-Cresol<br>Ortho-Cresol |
| Jones, 1952. | Gasterosteus aculeatus L.<br>Phoxinus phoxinus L.<br>Salmo trutta L. | $O_2$ |
| Jones, *et al* 1956. | Oncorhynchus kisutch<br>Oncorhynchus tshawytscha<br>Salmo gairdneri | Sulfate Waste |
| | Oncorhynchus kisutch<br>Oncorhynchus tshawytscha | Sulfite Waste |
| Bishai, 1962a. | Salmo salar<br>Salmo trutta | $O_2$ |
| Bishai, 1962b. | Salmo salar<br>Salmo trutta | pH |
| Sprague, 1964. | Salmo salar | Copper sulfate<br>Zinc sulfate |
| Sprague *et al*., | Salmo salar L. | Copper<br>Zinc<br>Copper + Zinc |
| Sprague, 1968. | Salmo gairdnerii | Zinc sulfate |

TABLE 1 - (Continued).

| Reference | Species | Chemical |
|---|---|---|
| | Steep Gradients (cont.) | |
| Hill, 1968. | Chologaster agassizi | $O_2$ |
| Sprague and Drury, 1969. | Salmo gairdnerii | Phenol<br>Chlorine<br>Alkyl benzene<br>Sulphonate |
| | Salmo gairdnerii<br>Salmo salar | BKME |
| Hansen, 1969. | Cyprinodon variegatus | DDT<br>Endrin<br>Dursban<br>Malathion<br>Sevin<br>2,4-D |
| Kleerekoper *et al.*, 1970. | Carassius auratus | $CuCl_2$ |
| Whitmore *et al.*, 1970. | Lepomis macrochirus<br>Micropterus salmoides<br>Oncorhynchus kisutch<br>Oncorhynchus tshawytscha | $O_2$ |
| Hansen, 1972. | Gambusia affinis | DDT<br>Malathion |
| Kleerekoper *et al.*, 1973 | Carassius auratus | $CuCl_2$ + Temperature |
| Bogardus *et al.*, (unpublished manuscript) | Notropis blennius<br>Notropis volucellus<br>Pimephales vigilax | Monochloramine |
| Fava and Tsai, 1976. | Rhinichthys atratulus | TRC, FRC, and CRC. |

TABLE 1 - (Continued).

| Reference | Species | Chemical |
|---|---|---|
| | <u>Fluviarium Methods</u> | |
| Hoglund, 1951. | Leuciscus rutilis | $O_2$<br>Nickel<br>Nitrate<br>Ferric-Nitrate |
| Lindahl and Marcstrom, 1958. | Leuciscus rutilus | 2,4,6-Trinitrophenol |
| Hoglund, 1961. | Coregonus nasus<br>Esox lucius<br>Gasterosteus oculeatus<br>Leuciscus idvarus<br>Leuciscus rutilus<br>Perca fluviatillis<br>Tinca tinca<br>Salmo alpinus<br>Salmo fontinalis<br>Salmo salar<br>Salmo trutta | Sulphite Waste Liquor |
| | Gasterosteus aculeatus<br>Leuciscus rutilus<br>Salmo alpinus<br>Salmo fontinalis<br>Salmo salar<br>Salmo trutta<br>Tinca tinca | HCl |
| | Leuciscus rutilus<br>Salmo salar | $O_2$ |

subsequently modified by Wells (1915). Additional outlets at the center drain prevented the formation of vertical gradients and a series of stop-cocks was placed along the bottom of the trough to facilitate the removal of water samples.

Ishio (1964) developed a method that eliminated vertical gradients and allowed more than one fish to be tested simulataneously. The tank was divided into an upper and lower path by a horizontal layer of sand. Tap water was pumped into the lower path and toxicant was introduced into the upper path. Passage of the tap water upward through the sand diluted the toxicant and created a shallow horizontal gradient. Vertical gradients were eliminated by a perforated tube buried in the sand that aerated the entire length of the tank. The movement of twelve fish was monitored in the upper path to obtain a control, followed by introduction of the toxicant. The average position of the fish was plotted against the toxicant to determine the exact concentration releasing an avoidance response.

The second major type of avoidance study, the method that more sharply separates water and toxic solutions, was first proposed by Jones (1947). His apparatus consisted of a 59 cm plexiglass tube with inlets at both ends and central drains. In a control run, untreated water flowed into the tube at both ends and exited through a central drain. A fish was introduced at the open end of the apparatus, allowed 10-15 minutes to adjust to the experimental procedure, and then monitored in the trough every 30 seconds for 10 or 15 minutes. After the control run was completed, a test solution replaced the water at one end of the trough and fish movements were recorded on graph paper for 7-120 minutes.

The above technique was simple, suitable for many applications, and has been used extensively (Jones, 1947, 1951, 1952; Bishai, 1962a, 1962b). The apparatus was further modified by Sprague (1964) for studying the effect of metal solutions on salmon. His plexiglass tube measured 114 cm in length and was drained by four central hoses to improve the separation of the two bodies of water. This basic design was used with few modifications by Sprague _et al_. (1965), Sprague (1968), Hill (1968), and Sprague and Drury (1969).

Jones _et al_. (1956) investigated the response of salmonids to industrial wastes with an avoidance trough consisting of a rectangular wooden trough that was partitioned at one end into four parallel channels. Water entered each channel from an inlet at the closed end and exited from a drain at the lower, open end. For each trial, two channels served as controls and the others were treated with a toxic solution. Fish had the option to pass from the lower half of the trough into any of the four upper troughs. After the fish began exploratory behavior, the number of entries into each channel was recorded. The apparatus of Bogardus _et al_. (unpublished manuscript) was very similar to Jones _et al_. (1956) except that only two separate parallel flowing bodies of water (one toxicant, another untreated) were created.

Kleerekoper (1967) described a tank with a undirectional flow of water and a large (5 x 5 x 0.5 m), open area for monitoring the response of fish to toxic gradients. Water entered through one entire wall, passed through baffles which produced an almost laminar flow and created a stable gradient in the open test area. Water exited by an overflow pipe along the opposite wall. Embedded in the floor of the test area were 1,936 photoconductive cells interfaced with a collimeter above the tank by a small computer. When a fish passed over a photocell, light from a collimeter was intercepted and a computer recorded the fish's position. Each experiment consisted of three consecutive eight hour recordings of the locomotion of a single fish with "clean" water initially in the entire tank, with toxicant introduced into one half of the tank, and then with toxicant introduced into the opposite half of the tank. Therefore, the time accumulated in either area of the tank, the average radius of each turn, and the orientation of the fish's movements were studied.

Westlake and Lubinski (1976) described a similar system that was more compact and inexpensive. Their preference-avoidance tank was 100 x 50 x 50 cm with a deep end divided into two equal sections and a shallow end housing the experimental fish. Water introduced in the deep end mixed, flowed through a baffle that laminated the flow and then entered the shallow experimental end. A test consisted of monitoring the fish for one hour in "clean" water and one hour after the toxicant was added. A portable television camera mounted directly over the observation area produced an electrical signal of variable voltage. The fish's body produced a drop in voltage which was processed by a small computer that recorded the location of the fish. In this procedure, sophisticated parameters of locomotion could be analyzed in addition to a simple avoidance response.

Höglund (1951) attempted to integrate both the shallow gradient and the steep gradient methods in a "fluviarium" that consisted of a stainless steel trough 250 cm long, 32 cm wide and 12 cm deep. This trough was subdivided into an apportionment box, a central section of nine vertical glass plates creating ten longitudinal sections, and a test chamber to house the fish. Toxic concentrations were created in the apportionment box. The longitudinal plates of glass reduced turbulence in the water and stabilized the concentrations so that fish in the holding tank could select the most favorable environment from a stable and reproducible series of concentrations. Fish behavior was observed and the number of appearances in each concentration was recorded. The sum of these appearances was presented in histograms to show which sections were avoided or preferred.

## DISCUSSION

The execution of any avoidance response presupposes the presence of a directive factor. Directive factors, as defined by Fry (1947) include all cues that enable an organism to determine its own location and the location of other organisms in the environment. In addition, avoidance behaviors depend upon the organism's ability to discriminate between two bodies of water, for without this perception, avoidance of lethal doses would not occur. Hasler and Wisby (1949) showed that a fish's ability to discriminate between different waters is very acute, but little research has been performed to determine the locus for toxicant reception in spite of its great importance to orientation and locomotion in fish.

Once the toxicant has been perceived by the fish, it may respond in several ways. The most simple response is a kinesis in which the animal's body is not oriented with respect to the source of stimulation. This stimulus may change the swimming speed (orthokinesis) or the rate of turning (klinokinesis) (Hinde, 1970). Taxes are more advanced movements directing an animal toward (positive taxis) or away from (negative taxis) a stimulant (Maier and Maier, 1970). A movement that is directed by alternate comparisons is termed a klinotaxis and is often found in animals that have only one receptor or several receptors that are widely dispersed. In this behavior, a fish will move its receptor into one solution and compare these sensations with those received in the alternate solution. After the comparison is made, the fish may orient itself either toward or away from either stimulus. These movements, which are successive in time and space, were clearly demonstrated by Collins (1952). The second taxes - tropotaxis - is initiated by a simultaneous comparison of different solutions usually involving symmetrical or bilateral sense organs. These movements are most successful in steep gradients and can be easily identified if one of a pair of receptors is destroyed (Bardach _et_ _al_., 1967).

It is difficult to separate the different orienting components in vertebrates, which has led to much debate regarding the mechanism that predominates in an avoidance response. Proponents supporting kinetic movements point to the erratic but energetic movements of fish that move into poorly oxygenated water (Jones, 1952). Dandy (1972) found that high chlorine concentrations (0.08 ppm) stimulated general activity in brook trout. Presumably these fish exposed to high levels of chlorine will move rapidly by chance into freshwater, where the response would subside. At certain low chlorine concentrations (0.04 ppm) brook trout activity was depressed. This was suggested as a possible cause for the preference behavior towards lethal substances reported by several workers, e.g. Sprague and Drury (1969). If the fish's activity is depressed, its chances of moving into a freshwater zone would be lessened. These ideas were refuted by Stott and Cross (1973) who concluded that directed actions dominated the response by roach to low oxygen conditions. They noted that low oxygen increased the fish's activity, but did not significantly alter their position in a shallow gradient because activity remained high even after the fish returned to the untreated water. Therefore, it may be assumed that both directed and undirected movements are normally present and contribute to a greater or lesser degree depending upon the fish species and the nature of the toxicant (Höglund, 1961).

Prospective investigators must consider the advantages and disadvantages of each method. The most obvious decision to be made is whether to create a shallow or a steep gradient. Shallow gradients have the distinct advantage of simulating the majority of field situations. In the immediate vicinity of an industry's discharge, chemical gradients are very steep. As it is diluted downstream, the discharge plume dissipates and a shallow gradient covers a much larger region of the stream. When fish swim up the gradient from downstream locations, they are subjected to ever increasing toxic concentrations. The use of shallow gradients also presents several problems: (1) shallow gradients are difficult to reproduce with accuracy; (2) the threshold avoidance concentration is difficult to ascertain in a continuous gradient unless it lies directly at a sampling point; (3) fish placed in a shallow concentration gradient probably select a favorable location as opposed to avoiding a certain toxic concentration; and (4) most importantly, shallow gradients cannot provide directional cues for fish. Therefore, taxes are not successful in such gradients. A single toxic concentration allows the fish's sense organs the best opportunity to discriminate between two opposing bodies of water, which represents the greatest advantage of steep gradients. These gradients provide the necessary cues for successful directed movements such as klinotaxes and tropotaxes. The chief objection to steep gradients concerns their applicability to field situations. Steep gradients are not particularly common in nature but are present at the confluence of two bodies of water or at an industry's outfall.

The parameter chosen as an index of avoidance behavior must be compatible with the physiological and behavioral responses of each species to each toxicant. Shelford and Allee (1914) recorded the number of entries into the modified water as a measure of avoidance behavior. Whitmore _et al_. (1970) placed markers at varying distances within the experimental solution to determine the depth of penetration for each visit. In both cases, it was assumed that higher concentrations of toxicant would elicit a greater turning frequency and consequently more shallow visits into the treated water. These entry indices account for klinokinetic movements which result in more frequent turns in the treated water and for klinotaxes in which the fish must repeatedly enter the treated water to compare these sensations with those in untreated water. Unfortunately, these methods do not account for orthokinetic responses in which the fish move deeply into the toxicant but leave so quickly that less time is actually spent in the modified side. This may account for contradictory opinions that entry indices are adequate (Kleerekoper _et al_., 1970) and inadequate (Fava and Tsai, 1976) measures of avoidance behavior. Elapsed time may be the most accurate measure of both kinetic and taxic behavior (Fava and Tsai, 1976). Time indices account for increases in swimming speed as fish move more rapidly through a toxicant and for increased turnings as fish avoid unfavorable conditions. The measurement of other parameters does not provide such complete information.

# III. MATERIALS AND METHODS

All avoidance trials were conducted in a mobile laboratory on the site of Appalachian Power Company's Glen Lyn power plant. This laboratory, described in detail by Cherry *et al*. (1974), houses a temperature preference unit, an avoidance unit and a series of holding tanks to maintain each fish species at several acclimation temperatures. Water pumped directly from the New River supplied all apparatuses within the laboratory. The chemistry of this water is presented in TABLE 2.

## FISH

Golden shiner (*Notemigonus crysoleucas*, Mitchill), carp (*Cyprinus carpio*, Linnaeus), and channel catfish (*Ictalurus punctatus*, Rafinesque) were used for experimentation. The carp are primarily detritivores and the golden shiner provide forage for predators such as the channel catfish. These fish are found in the New River (Stauffer *et al*., 1974) and the toxicity of free and combined residual chlorine to these species has also been determined (Heath, in press). All fish were purchased from a commercial warm-water hatchery in Windsor, Virginia because they were not available in sufficient quantities from the New River throughout the year. Upon arrival at the laboratory, fish were examined for external parasites, and if necessary, treated with oxytetracycline (Stephen, 1975). Shortly thereafter, they were divided among several 940-liter holding tanks and acclimated at five temperatures - 6, 12, 18,

TABLE 2 - New River water chemistry for 1975. Summary of 263 samples ( Mean ± standard deviation).

| Parameter Measured | Concentration |
|---|---|
| Alkalinity | 46.5 ± 5.7 mg/liter |
| Hardness | 66.4 ± 8.0 mg $CaCO_3$/liter |
| pH | 7.82 ± 0.2 |
| Turbidity | 55.2 ± 65.1 JTU |
| Conductivity | 135.1 ± 19.9 micromho |
| $PO_4$ | 0.622 ± 0.449 mg/liter |
| $NO_3$ | 3.38 ± 0.91 mg/liter |
| Dissolved Oxygen | 10.5 ± 1.8 mg/liter |
| Suspended Solids | 9.69 ± 5.37 mg/liter |

24, and 30 C for a minimum of one week before being used in avoidance experiments. Allen and Strawn (1971) found that channel catfish approach total temperature acclimation in less than one week and it was assumed that the acclimation rates of the carp and golden shiner are similar.

## CHLORINE CHEMISTRY

A stock chlorine solution used in all avoidance trials was prepared by dissolving 1.667 grams of calcium hypochlorite in one liter of water. This compound disperses in water to yield hypochlorite ion as follows:

$$Ca(OCl)_2 \longrightarrow Ca^{++} + 2(OCl^-) \qquad \text{Equation 1}$$

which immediately equilibrates with hydrogen ions to yield hypochlorous acid (Sawyer and McCarty, 1967):

$$Ca^{++} + 2(OCl^-) + H_2O \longrightarrow Ca(OH)_2 + 2HOCl$$

Equation 2

The hypochlorous acid readily enters into various side reactions with reducing agents or organic compounds which must equilibrate before residual chlorine is available as a biocide for disinfection purposes. The difference between the applied chlorine concentration and the chlorine demand is termed the total residual chlorine. After the chlorine demand is satisfied, the hypochlorous acid reacts with free ammonia to form monochloramines, dichloramines and trichloramines, collectively termed the combined residual chlorine or CRC:

$$NH_3 + HOCl \longrightarrow NH_2Cl + H_2 \qquad \text{(monochloramines)}$$

$$NH_2Cl + HOCl \longrightarrow NHCl_2 + H_2O \qquad \text{(dichloramines)}$$

$$NHCl_2 + HOCl \longleftrightarrow NCl_3 + H_2O \qquad \text{(trichloramines)}$$

The relative proportions of each CRC constituent are largely dependent on pH and time for reaction to occur (Baker, 1959).

The hypochlorous acid which does not bind with ammonia tends to undergo partial dissociation to produce hypochlorite ion and hydrogen ion:

$$HOCl \rightleftharpoons H^+ + OCl^- \qquad \text{Equation 3.}$$

The acid's dissociation constant (Ka) is largely governed by the water temperature in degrees Kelvin (Morris, 1966):

$$pKa = \frac{2500}{K} - 6.8018 + (0.01998)\,K \qquad \text{Equation 4.}$$

The percent hypochlorous acid can be calculated if the dissociation constant (Ka) and the hydrogen ion concentration ($H^+$) of the water are known (White, 1972):

$$\%\ HOCl = \frac{1}{1 + Ka/H^+} \qquad \text{Equation 5.}$$

The hypochlorite ion plus hypochlorous acid comprise the free residual chlorine (FRC). Finally, TRC = CRC + FRC.

## AVOIDANCE DEVICES

The avoidance apparatus consisted of a water/toxicant delivery system, two experimental troughs and a monitoring system (FIGURE 1). The main components of the delivery system were two 60-liter circulating water baths that adjusted the ambient water temperature to the appropriate acclimation temperature. These baths pumped the water into opposite ends of each trough at approximately 600 ml/min. The avoidance troughs (1.9 m long x 20.5 cm wide x 14 cm deep), with four drains at their midpoint, created a sharp demarcation between the waters supplied at either end of each trough. These drains were adjustable so that the water depth within each trough could be altered to suit individual fish. The troughs were isolated from the remainder of the laboratory with plywood sheathing. A television camera, connected to a closed circuit monitor and mounted within the enclosure, allowed for observations of the troughs without alarming the fish.

Water samples, siphoned from the troughs by several tygon tubes (FIGURE 2), were analyzed with a Wallace-Tiernan amperometric titrator for total residual chlorine and free residual chlorine as directed by Standard Methods (APHA, 1971). This procedure is preferable to the starch/iodide titrations which have an indefinite and transitory endpoint (Heukelekian *et al.*, 1953) and the orthotolidine method which is not suitable in turbid waters (Collins and Deaner, 1973). The hydrogen ion concentration in sample water was determined with a Corning Model 610-A portable pH meter.

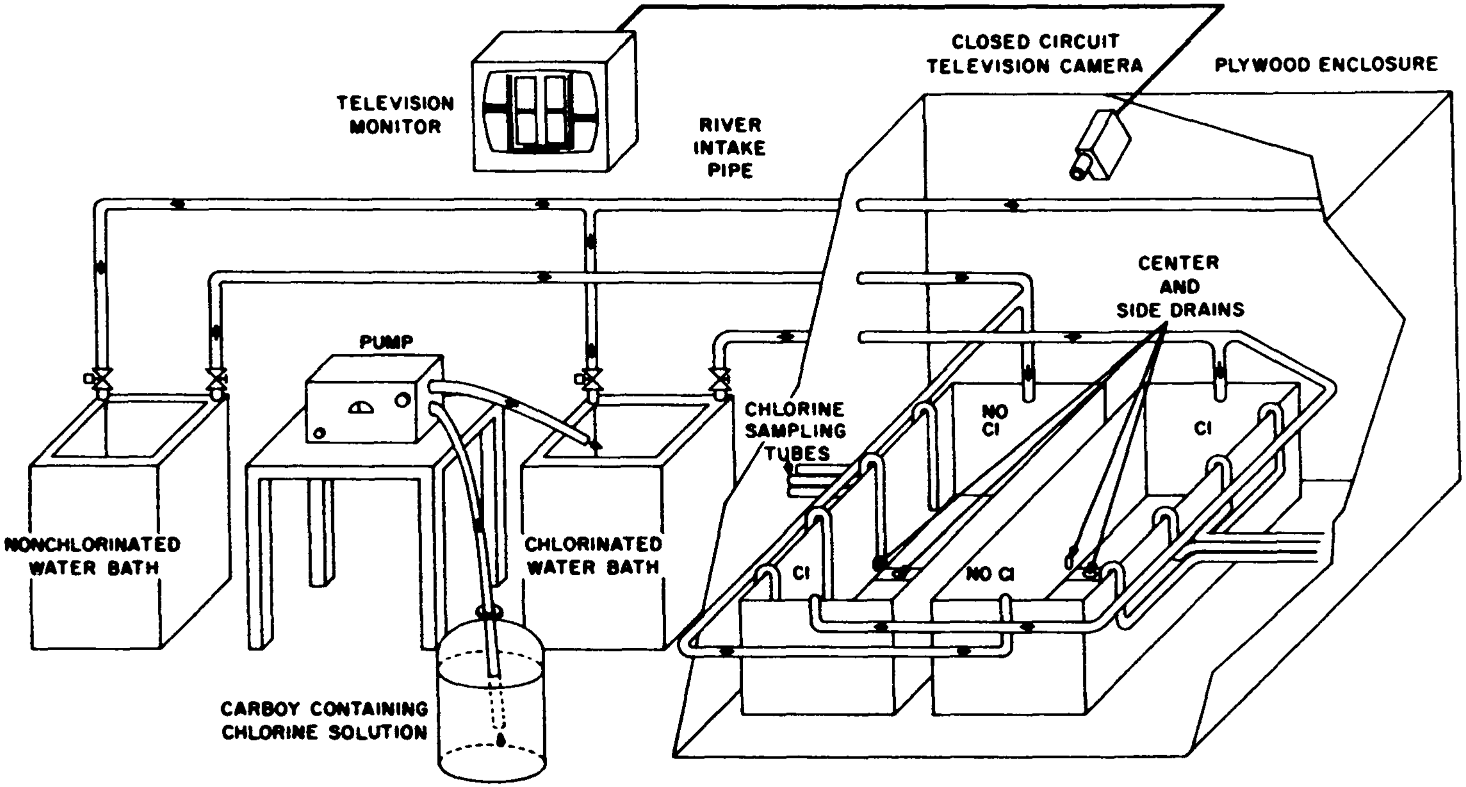

FIGURE 1 - Schematic diagram of the avoidance apparatus.

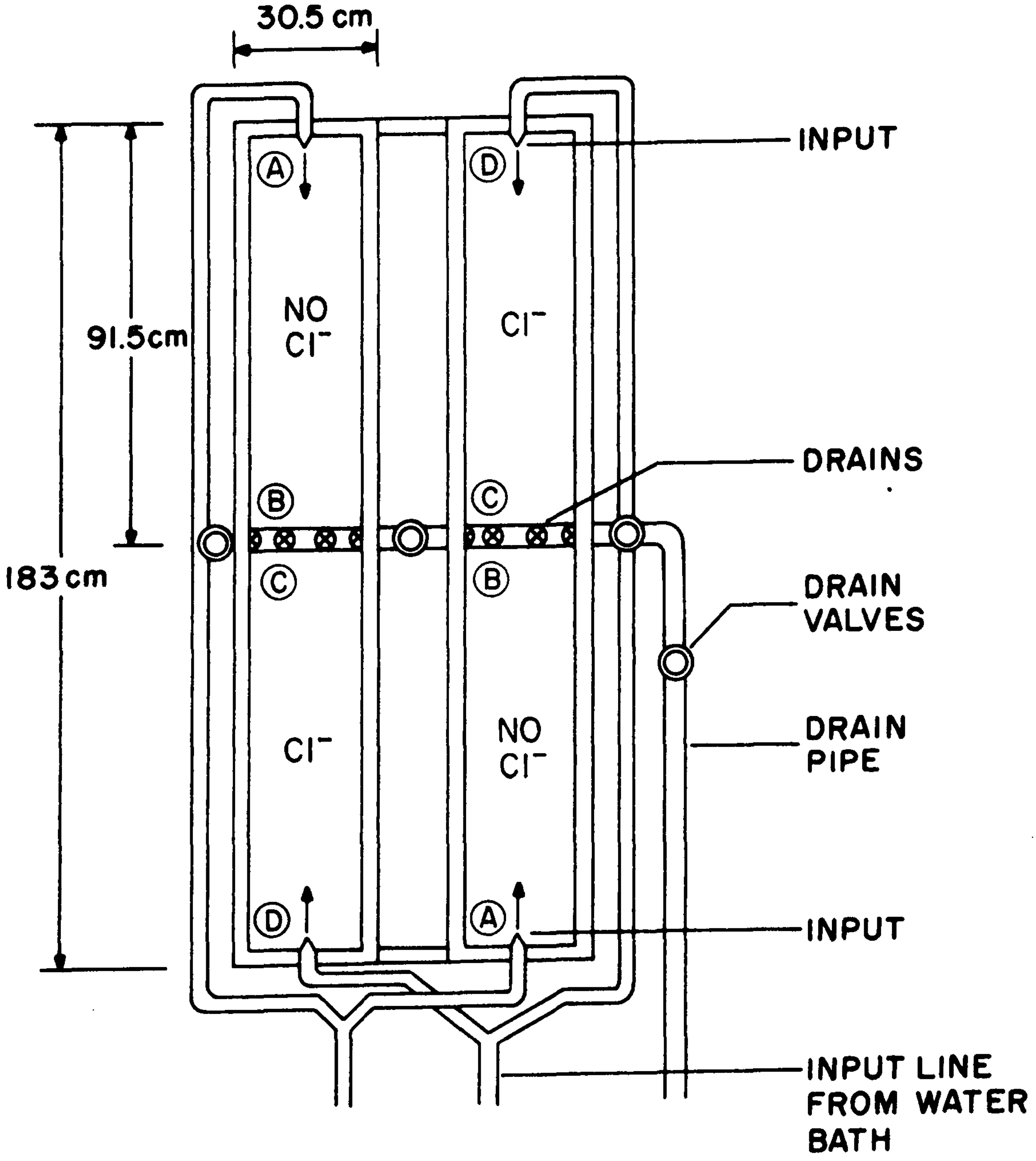

FIGURE 2 - Diagram of the experimental troughs and the sampling points.

## CHLORINE AVOIDANCE EXPERIMENTS

One fish was placed in each trough after the troughs were filled to a depth of 2-3 cm. The fish were allowed time to resume normal exploratory behavior, generally about 20-60 minutes, and then the number of seconds spent on each side of the central drain was recorded for a ten-min period. These observations, in which the water quality was the same throughout the troughs, comprised the control data.

The stock chlorine solution was then added to one water bath in sufficient quantities to satisfy the water's chlorine demand and produce 0.025 mg/liter TRC in one half of each trough. After this chlorine slug reached the drain in each trough, a steep chlorine gradient formed and the number of seconds spent in the treated water was recorded for another ten-min period. A concentrated chlorine solution in a carboy was fed directly into the chlorinated water bath with a peristaltic pump, to maintain a constant TRC throughout the trial. At the end of each observation period the residence time, TRC, FRC, and pH were tabulated. The same fish were tested at successively doubling doses of 0.050, 0.100 .... 0.400 mg/liter TRC. Higher residuals were obtained by adding additional stock chlorine to the treated water bath and carboy. Golden shiners were also tested at 0.800 mg/liter TRC. After a trial was completed, the fish were measured (fork length), and the water baths and experimental troughs were drained and cleaned to remove all traces of residual chlorine. A minimum of eight fish per species were tested at each of five acclimation temperatures - 6, 12, 18, 24, and 30 C.

## TEMPERATURE PREFERENCE TRIALS

Temperature preferenda at 12 and 24-C acclimation temperatures were obtained for all three species in a shallow horizontal temperature gradient described by Cherry *et al*. (1975). Briefly, the preference unit consisted of an epoxy-coated stainless steel trough (363 cm long, 36 cm wide, and 20 cm deep) with a battery of 12-250 watt infrared lamps arranged underneath it. Cold water from a circulating water bath was introduced at one end of the trough and gradually heated by the lamps at increasing intensity as it flowed towards a drain at the trough's opposite end, creating a shallow, horizontal temperature gradient. To restrict vertical movement, water depth was varied from 2.5 to 6.3 cm depending upon the size of the fish tested. The trough was enclosed by plywood sheathing but could be observed on a mirror mounted at an angle above the trough.

Four fish were used for each trial, and each test was duplicated at each acclimation temperature. Fish were placed in the trough at the point corresponding to their acclimation temperature, allowed to orient for 20 min (or as much as 60 min for slower reacting species) and then their position relative to the nearest immersed thermistor was recorded at two min intervals for 40 min. The mean temperature from all 20 observations was considered to be the selected temperature.

## COMBINED TEMPERATURE PREFERENCE/CHLORINE AVOIDANCE EXPERIMENTS

These experiments were identical to regular chlorine avoidance experiments except that fish were subjected to the additional parameter of temperature. Following the control runs (conducted exactly as before) but before chlorine was added, the water in one bath was heated to the temperature preferred by that species at the corresponding acclimation temperature, creating a steep thermal gradient within each trough. The fish were monitored for a 10 minute period and the number of seconds spent in the heated *vs* the unheated water was recorded. Chlorine was then added to the heated water and the same fish were tested through successive TRC concentrations of 0.025, 0.050, 0.100, 0.200 and 0.400 mg/liter. Samples were removed at the beginning and end of each recording period and analyzed for TRC, FRC and pH as before.

## STATISTICAL ANALYSIS

After recording the raw data as the number of seconds spent in the treated water, the values were divided by 600 to obtain a percent residence time in the treated water. Data were tested for normality graphically and tested for homoscedasticity with Bartlett's Test for Homogeneity of Variances (Sokal and Rohlf, 1969). The variances were found to be heteroscedastic and nonparametric statistics were employed. Each species and acclimation temperature was tested by the

Wilcoxon Sign Rank Test (Wilcoxon and Wilcox, 1964) for differences in the mean percent residence time among chlorine and/or temperature treatments. Statistical avoidance occurred when significantly less time (P=0.05) was spent in the treated water than during the control run.

In each trial, the experimental TRC concentration was adjusted until it closely approximated the target TRC. The CRC, FRC and HOCl residuals were not fixed by the experimental design and varied daily with changes in the pH, temperature and ammonia content of the river water. For example, for golden shiners acclimated at 30 C and tested at 0.400 mg/liter TRC, the FRC varied from 0.320 mg/liter on July 2 to 0.180 mg/liter on July 3. To negate much of the variation due to water quality fluctuations, the data were re-analyzed as if the CRC, FRC, or HOCl had been fixed effects. For example, the CRC concentrations varied from 0.00 to 0.490 mg/liter. This range was divided into eight equal subgroups, 0.060 mg/liter wide. The data were ranked, assigned to the appropriate subgroup and the rank sums tabulated. The data were tested for significant differences (P=0.05 level) in percent residence time between treatments with the Kruskal-Wallis Rank Sum Multiple Comparison Test (Hollander and Wolfe, 1973). This nonparametric procedure goes beyond the point of deciding whether the treatments are equivalent to the more important problems of selecting which, if any, treatments differ from each other. Because of the large number of records for each species and acclimation temperature and because the data did not fit evenly into every artificial subgroup, the approximation by Dunn was employed:

$$H \neq H_o \text{ if } \left|R_u - R_v\right| \geq Z_{(\alpha/\left[K(K-1)\right])} \left(\frac{N(N+1)}{12}\right)^{\frac{1}{2}} \left(\frac{1}{n_u} + \frac{1}{n_v}\right)^{\frac{1}{2}}$$

for subgroups u and v where R=the rank sum, Z=the upper tail probabilities for the standard normal distribution, N=the total number of observations and n=the number of observations within a subgroup. Following the CRC analysis, this procedure was repeated for FRC and HOCl residuals over intervals of 0.060 and 0.040 mg/liter, respectively.

## AVOIDANCE INDICES

Experiments were carried out to determine the validity of alternate indices of avoidance behavior, besides the residence time index. The mechanics of these supplemental experiments were identical to the regular avoidance experiments. Following the control, golden shiners at 18 C were exposed to progressively doubling target chlorine concentrations from 0.050 to 0.400 mg/liter TRC. The number of entries into the treated water and the average time elapsed per entry into the treated water were recorded at each target concentration. Both indices were examined for differences between treatments and control data with the Wilcoxon Sign Rank Test.

## EXPOSURES TO SINGLE CHLORINE DOSES

Supplementary experiments were conducted with golden shiners at 18 C to determine the influence, if any, of the first few chlorine exposures on the response of the same fish at higher chlorine doses. Learning may occur from the earlier exposures or these interactions may influence behavior by exposing the fish to chlorine for longer periods of time. Eight fish were allowed to select between 0.000 and 0.050 mg/liter TRC, eight other fish were offered the choice between 0.00 and 0.100 mg/liter TRC, etc. TRC, FRC, and pH before and after each trial were tabulated and the percent residence times in the treated water were tested for differences between chlorine treatments with the Wilcoxon Sign Rank Test.

# IV. RESULTS

## CHLORINE AVOIDANCE EXPERIMENTS

The percent residence time in the chlorinated water generally decreased for each species and acclimation temperature as the TRC increased. Golden shiners, the most sensitive and responsive of the three species tested, spent less time in the treated water as the chlorine concentration was raised above 0.050 mg/liter (FIGURES 3-7). Golden shiners slightly prefer the 0.025 mg/liter TRC dose when tested at 12 and 24 C and the 0.050 mg/liter TRC dose at 18, 24, and 30-C acclimation temperatures. Although these residence times were greater, they were not significantly different from control data. Mean CRC, FRC and HOCl concentrations were calculated for each avoidance threshold - the lowest TRC concentration at which the percent residence time differed significantly from the control data (FIGURE 8, Appendix). The minimum avoidance threshold was 0.199 mg/liter TRC at the 18-C acclimation temperature, with residual concentrations for CRC, FRC, and HOCl of 0.112, 0.086, and 0.027 mg/liter, respectively. The maximum avoidance threshold occurred at 0.395 mg/liter TRC at 24 C with residual concentrations for CRC, FRC, and HOCl of 0.255, 0.139, and 0.045 mg/liter, respectively. Fish acclimated at 6 C moved poorly in the troughs during control and treated trials, and the data generated were highly suspect. The golden shiners avoided 0.430 mg/liter TRC with residual concentrations for CRC, FRC and HOCl of 0.078, 0.351, and

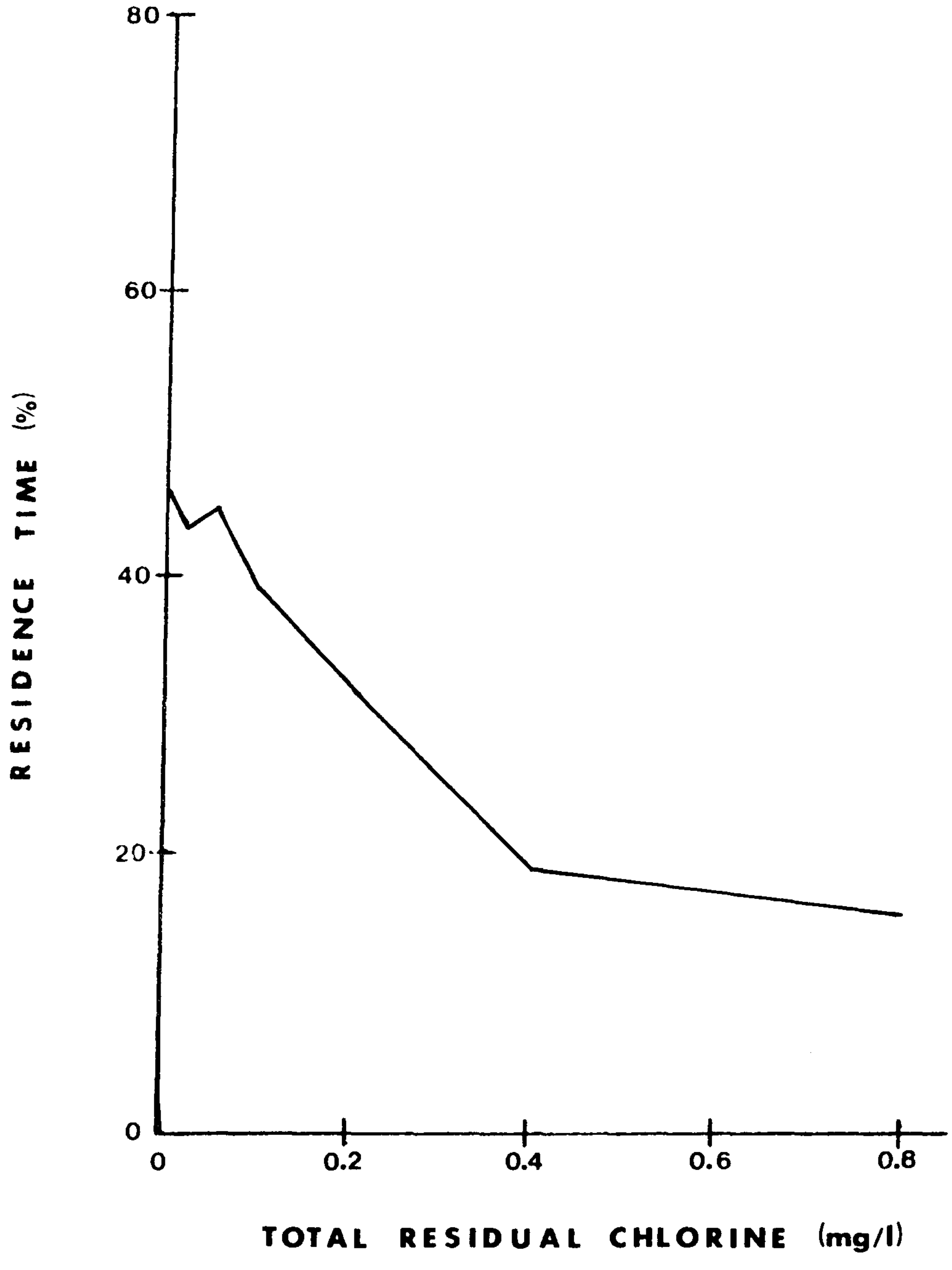

FIGURE 3 - Avoidance of TRC by golden shiners acclimated at 6 C.

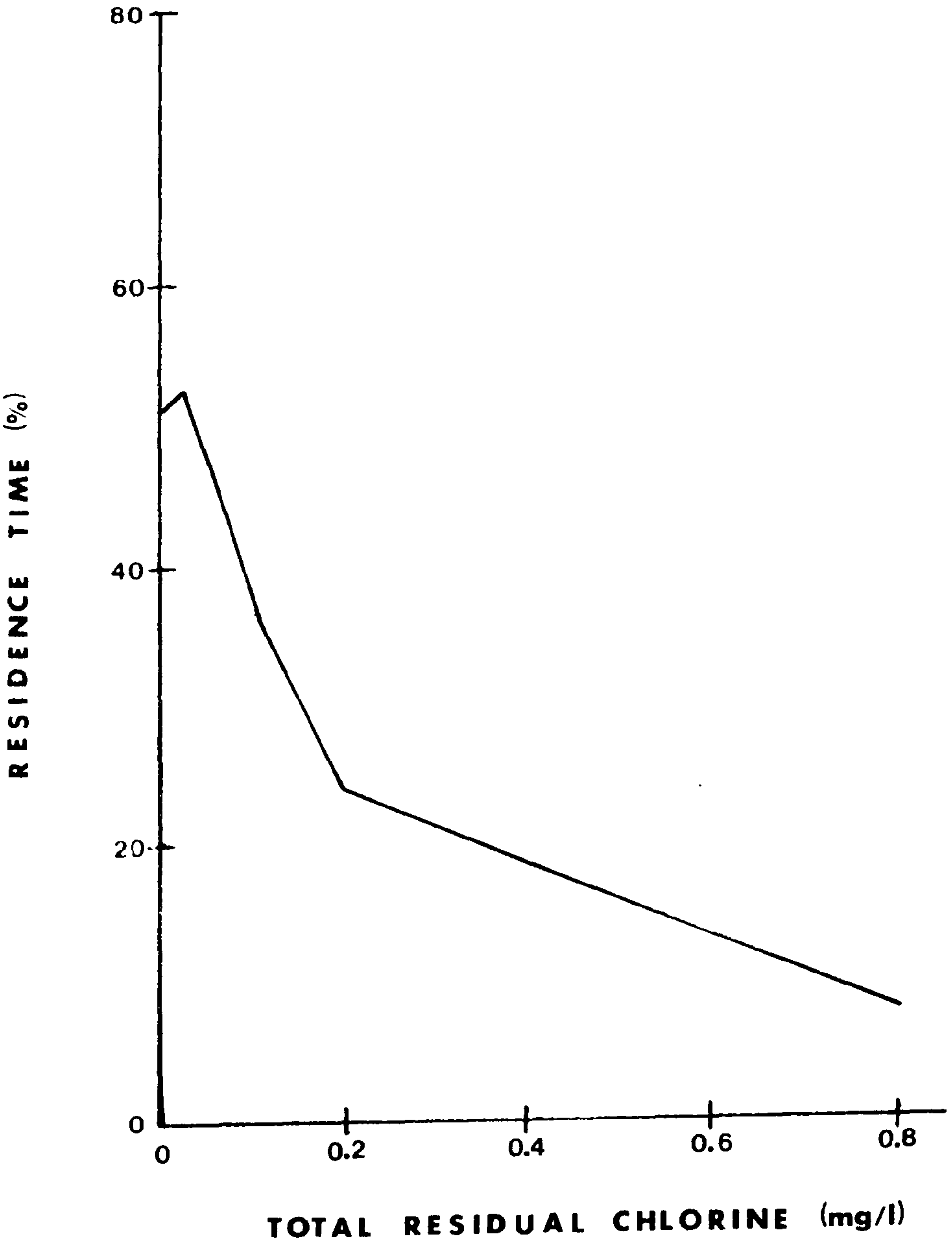

FIGURE 4 - Avoidance of TRC by golden shiners acclimated at 12 C.

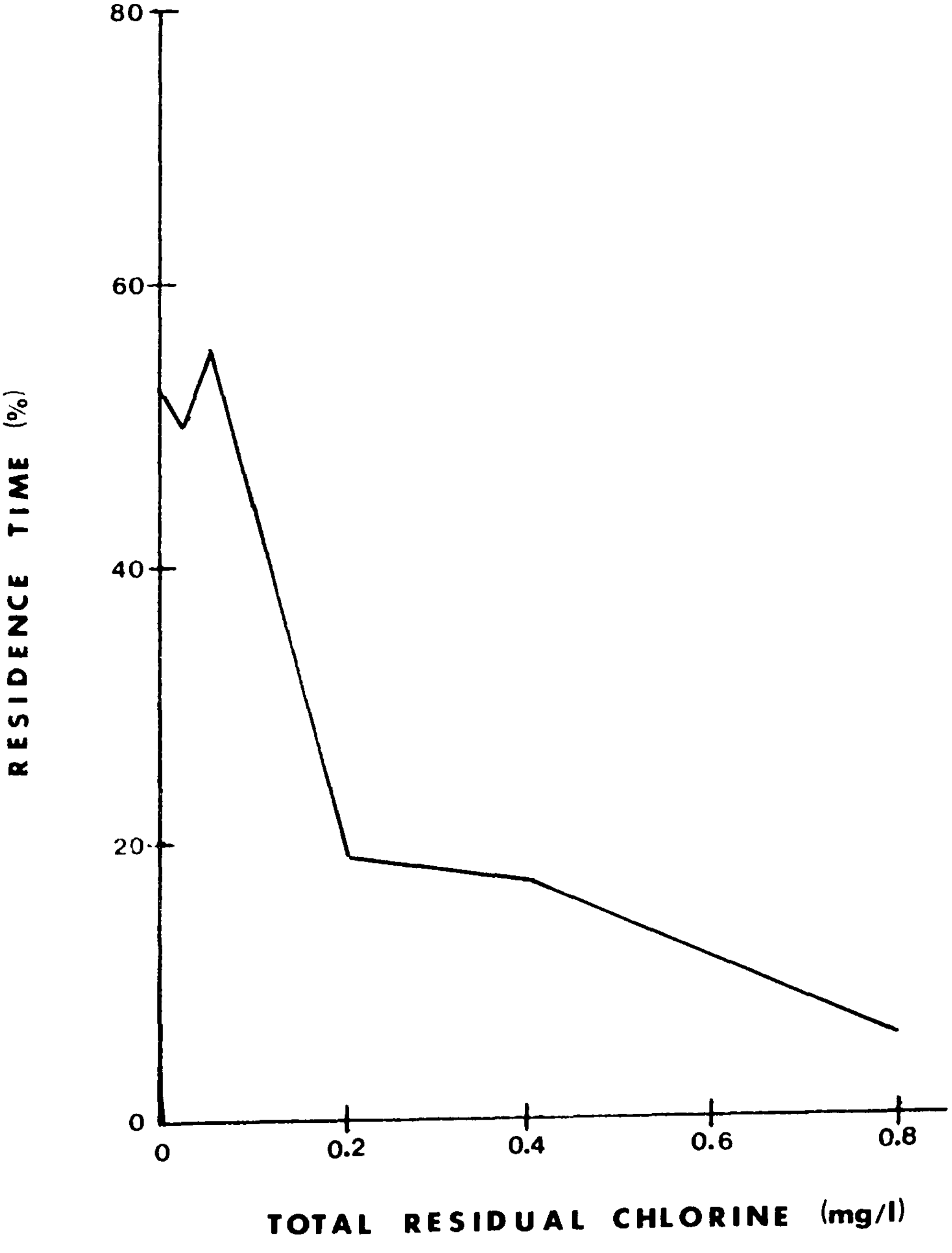

FIGURE 5 - Avoidance of TRC by golden shiners acclimated at 18 C.

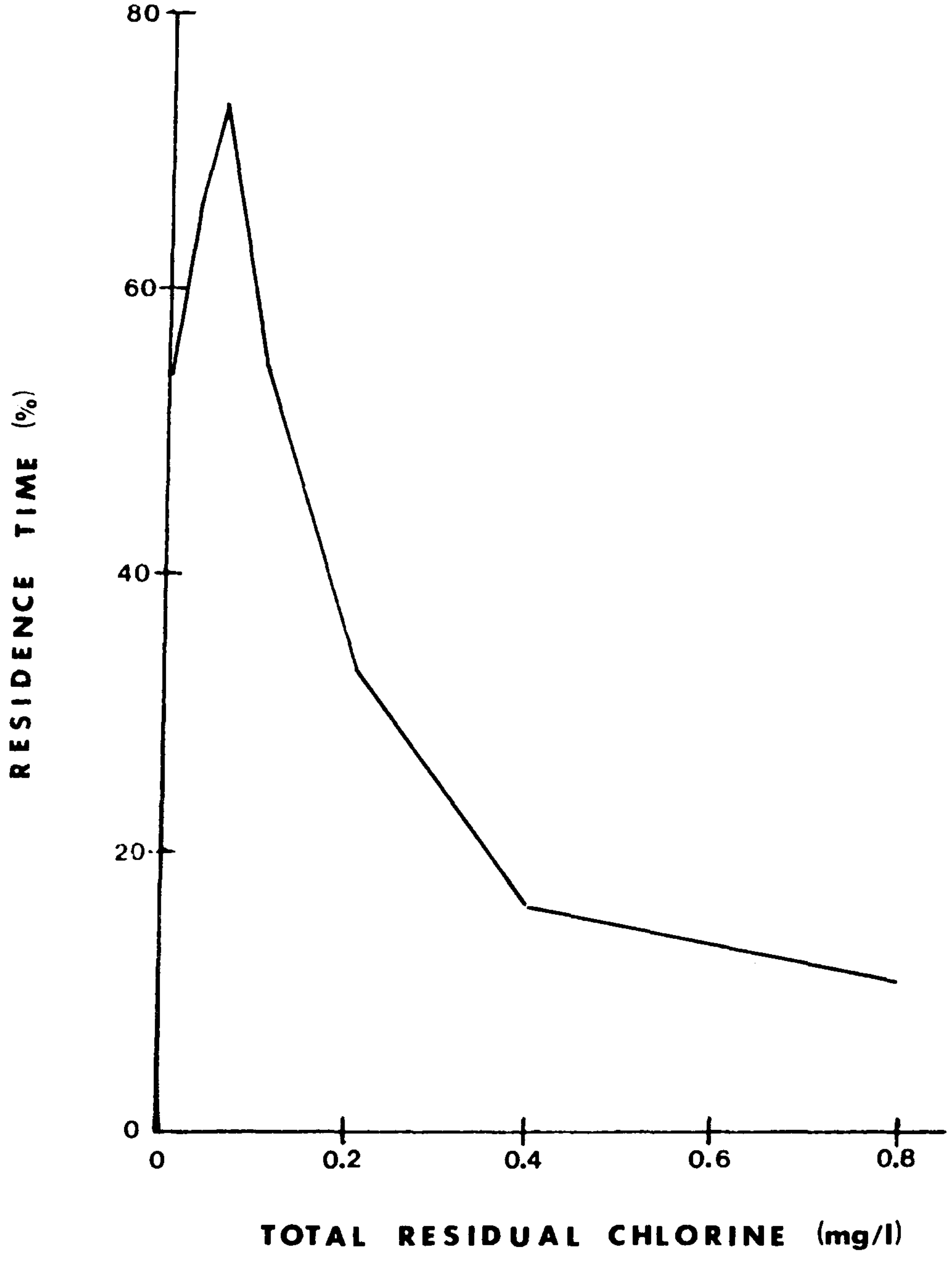

FIGURE 6 - Avoidance of TRC by golden shiners acclimated at 24 C.

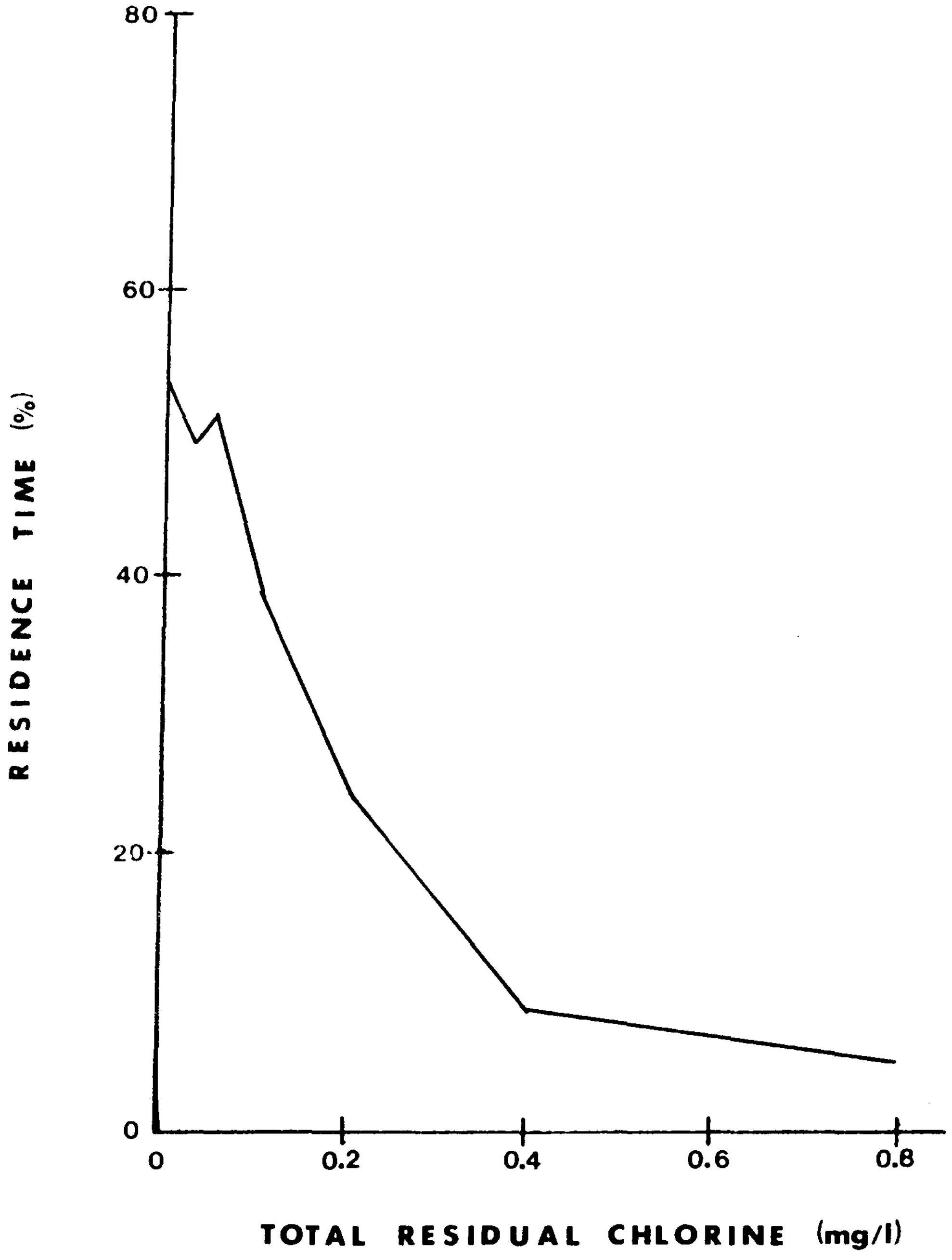

FIGURE 7 - Avoidance of TRC by golden shiners acclimated at 30 C.

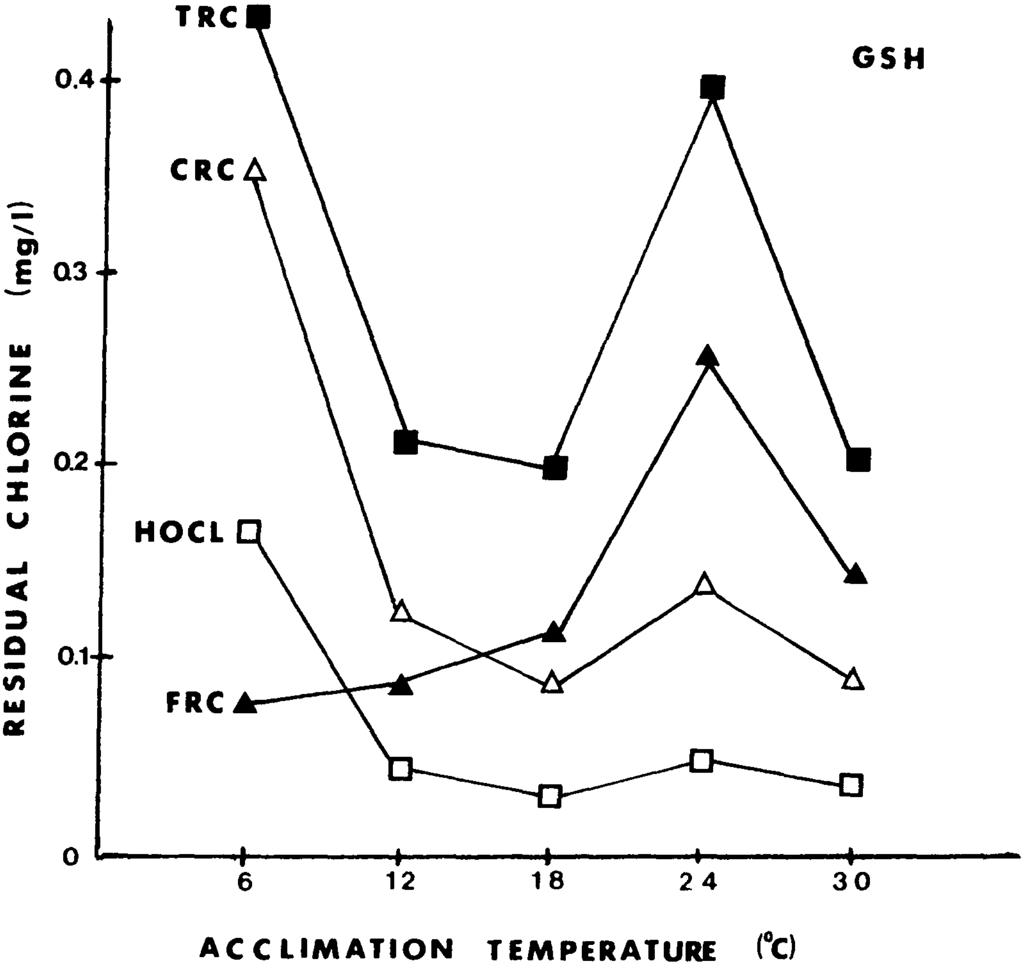

FIGURE 8 - Wilcoxon avoidance thresholds as a function of acclimation temperature for golden shiners.

0.163 mg/liter, respectively.

Channel catfish acclimated at 6 C moved poorly. Most had to be replaced frequently and did not avoid even high chlorine doses (FIGURE 9). At the higher acclimation temperatures, 12-30 C, the percent residence time generally decreased as the TRC concentrations were increased, except for a preference for 0.050 mg/liter TRC at 24 C (FIGURES 10-13). The minimum avoidance threshold of 0.205 mg/liter TRC occurred at 30 C with residual concentrations for CRC, FRC, and HOCl of 0.097, 0.108, and 0.037 mg/liter, respectively. The maximum avoidance threshold was 0.403 mg/liter TRC at 24 C with residual concentrations for CRC, FRC, and HOCl of 0.184, 0.219, and 0.066 mg/liter, respectively (FIGURE 14, Appendix).

The carp were sluggish at 6-C acclimation temperature but did avoid 0.233 mg/liter TRC with CRC, FRC and HOCl concentrations of 0.051, 0.182, and 0.091 mg/liter, respectively (FIGURE 15). At higher acclimation temperatures, the carp spent less time in the treated water as the TRC was raised (FIGURE 16-19), except at the treatment levels of 0.025 mg/liter TRC at 24 C and 0.050 mg/liter TRC at 18 and 24 C. At these treatment levels, preference for the treated water was evident. The minimum avoidance threshold of 0.104 mg/liter TRC occurred at 12 C with residual concentrations for CRC, FRC and HOCl of 0.056, 0.049, and 0.019 mg/liter, respectively. The maximum avoidance threshold of 0.212 mg/liter TRC occurred at 24 C with residual CRC, FRC and HOCl concentrations of 0.074, 0.138, and 0.035 mg/liter, respectively (FIGURE 20, Appendix).

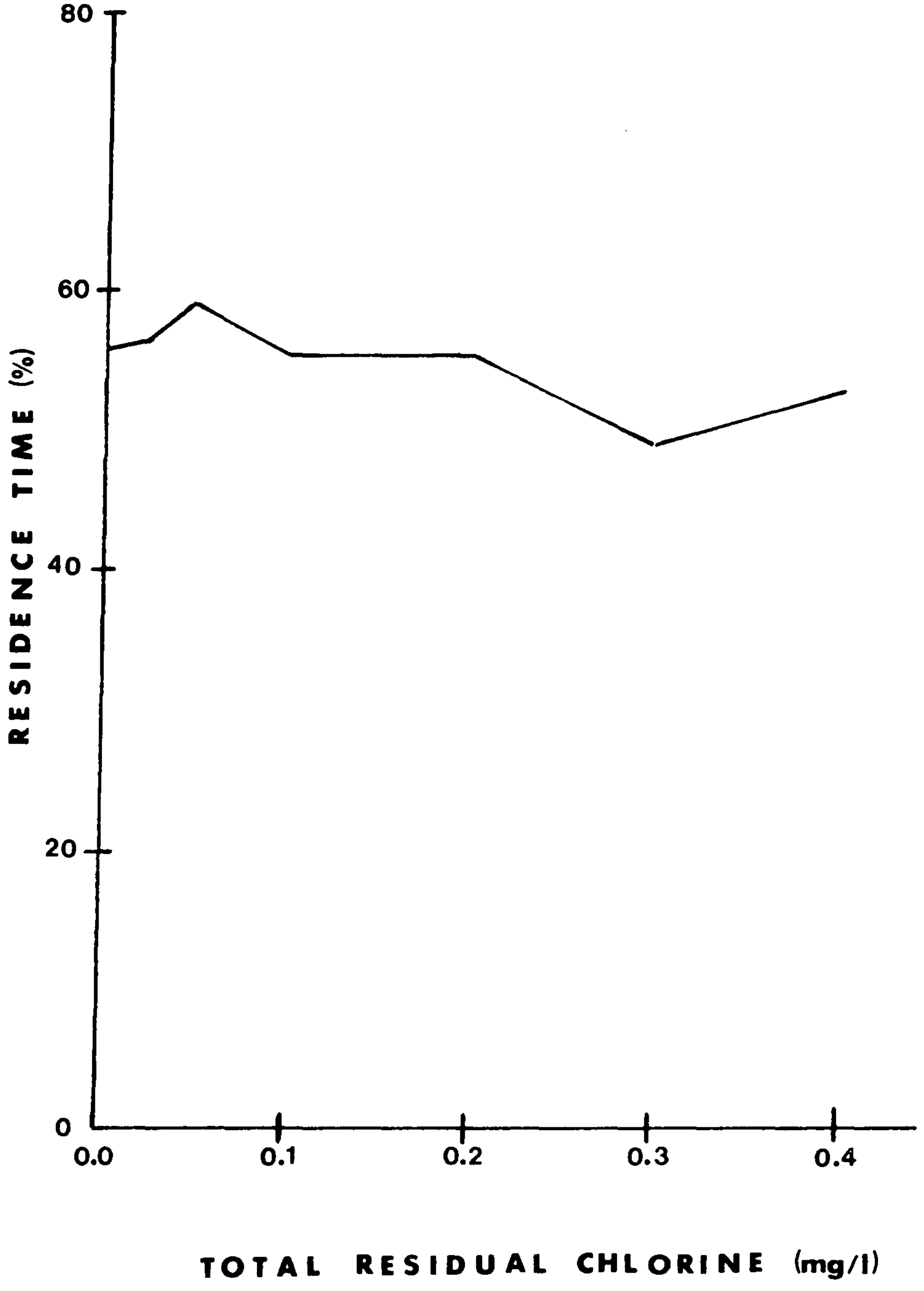

FIGURE 9 - Avoidance of TRC by channel catfish acclimated at 6 C.

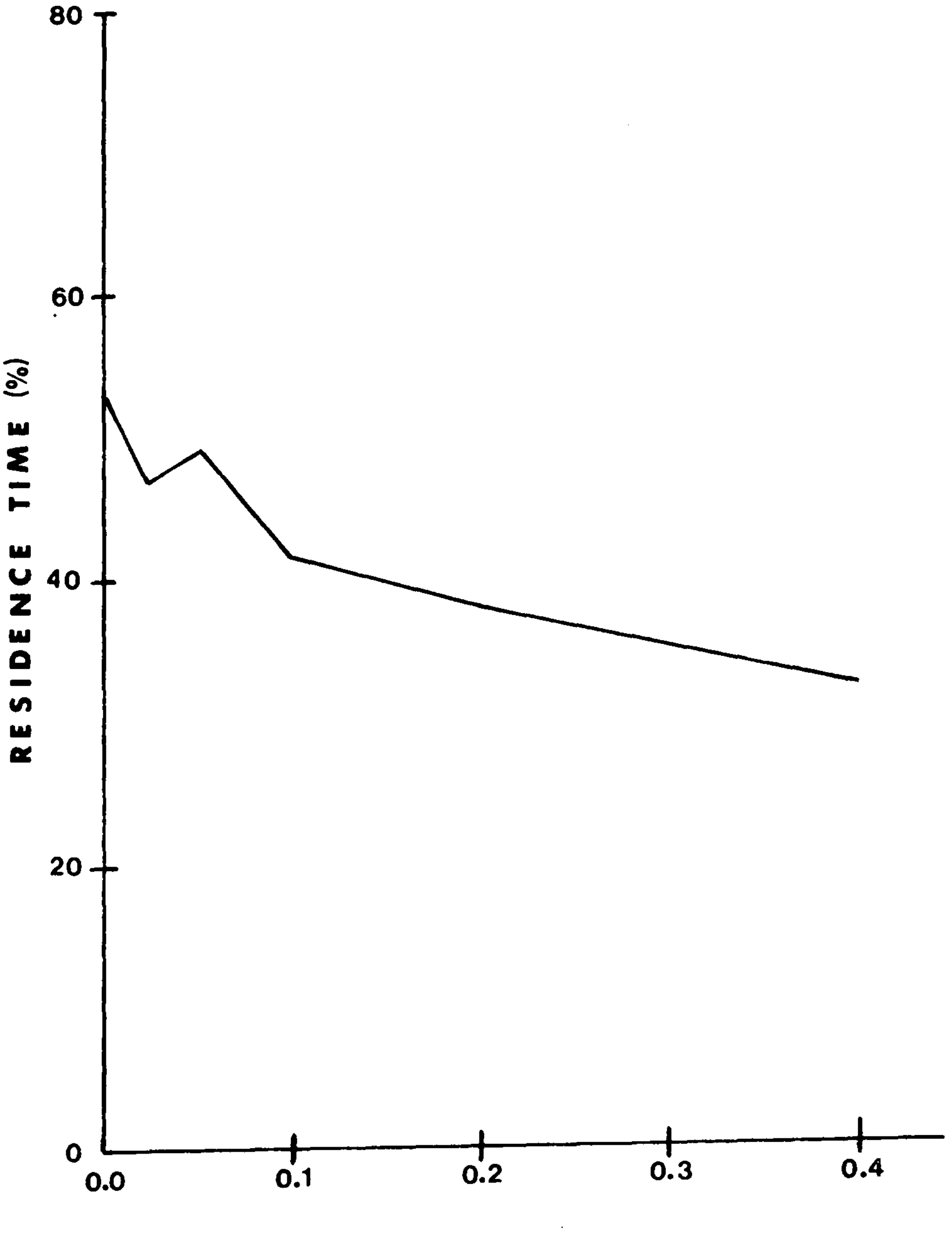

FIGURE 10 - Avoidance of TRC by channel catfish acclimated at 12 C.

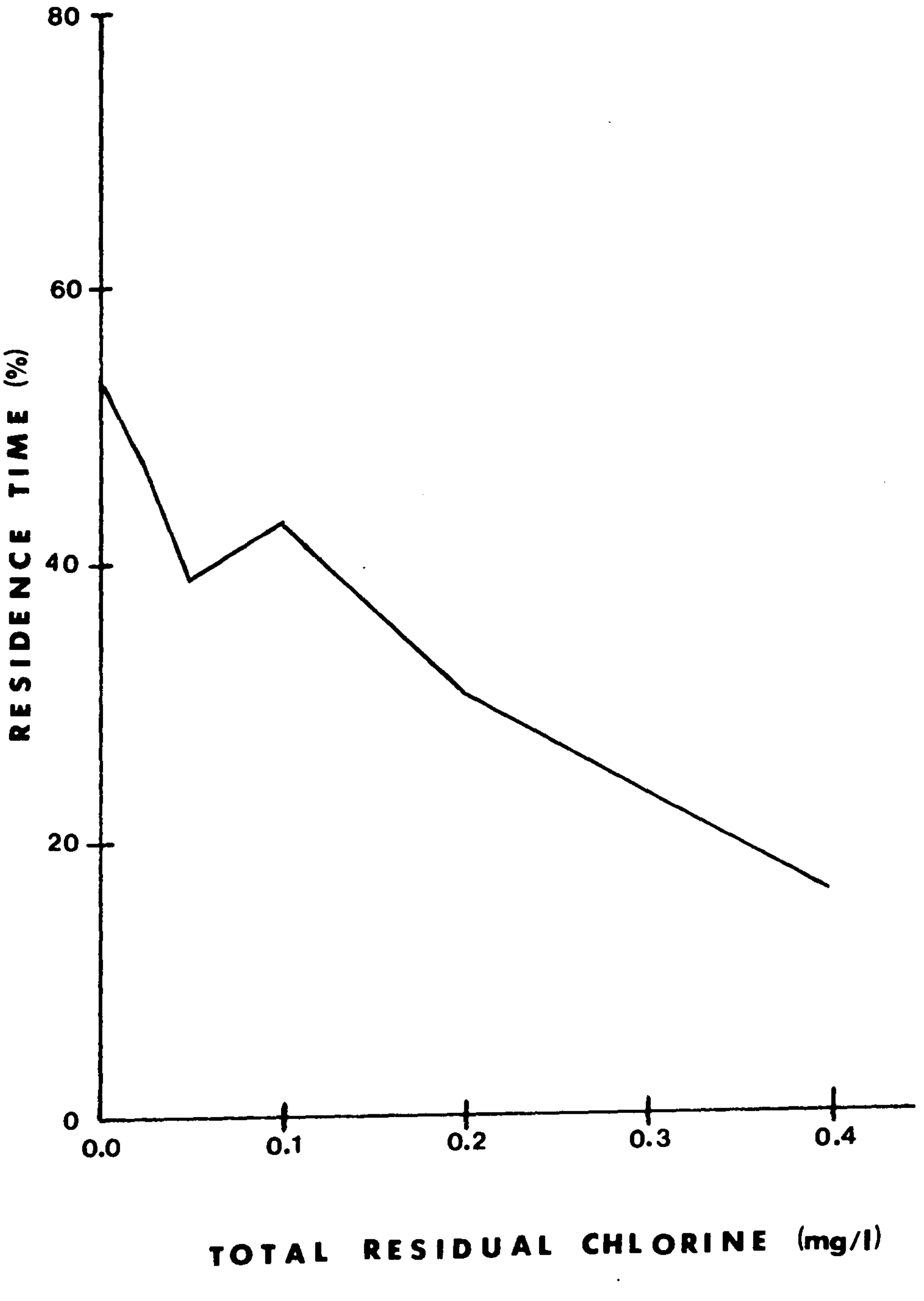

FIGURE 11 - Avoidance of TRC by channel catfish acclimated at 18 C.

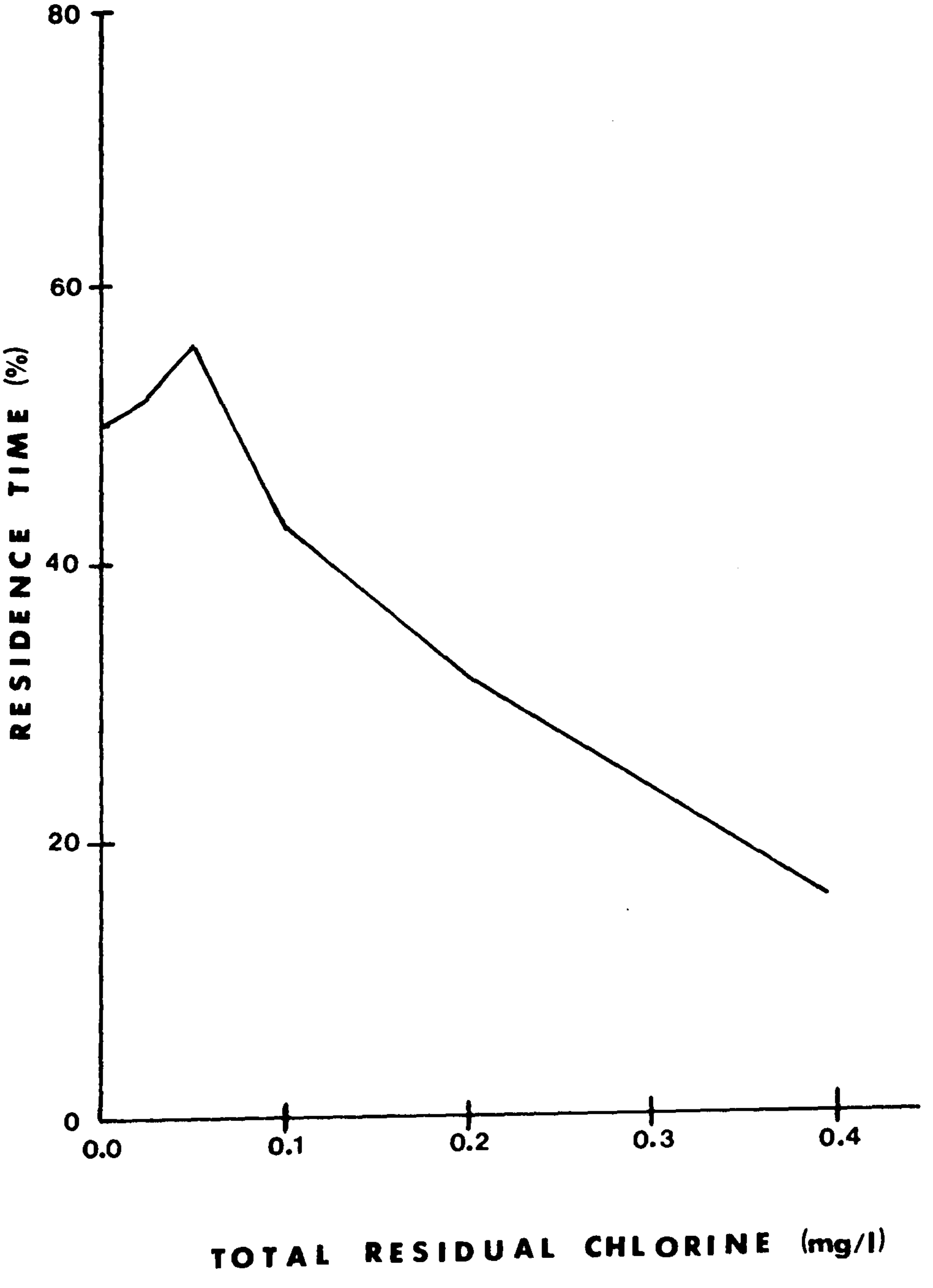

FIGURE 12 - Avoidance of TRC by channel catfish acclimated at 24 C.

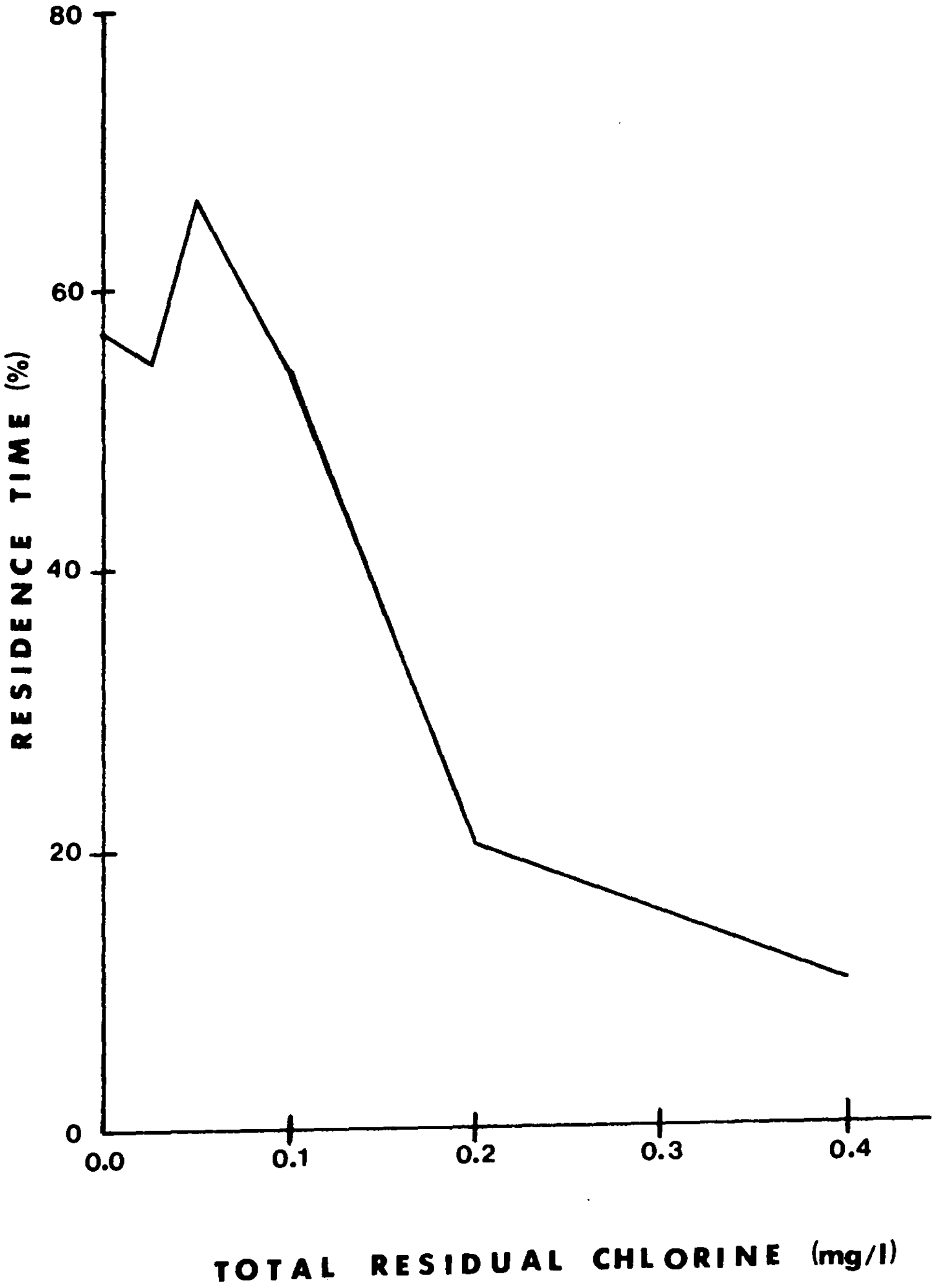

FIGURE 13 - Avoidance of TRC by channel catfish acclimated at 30 C.

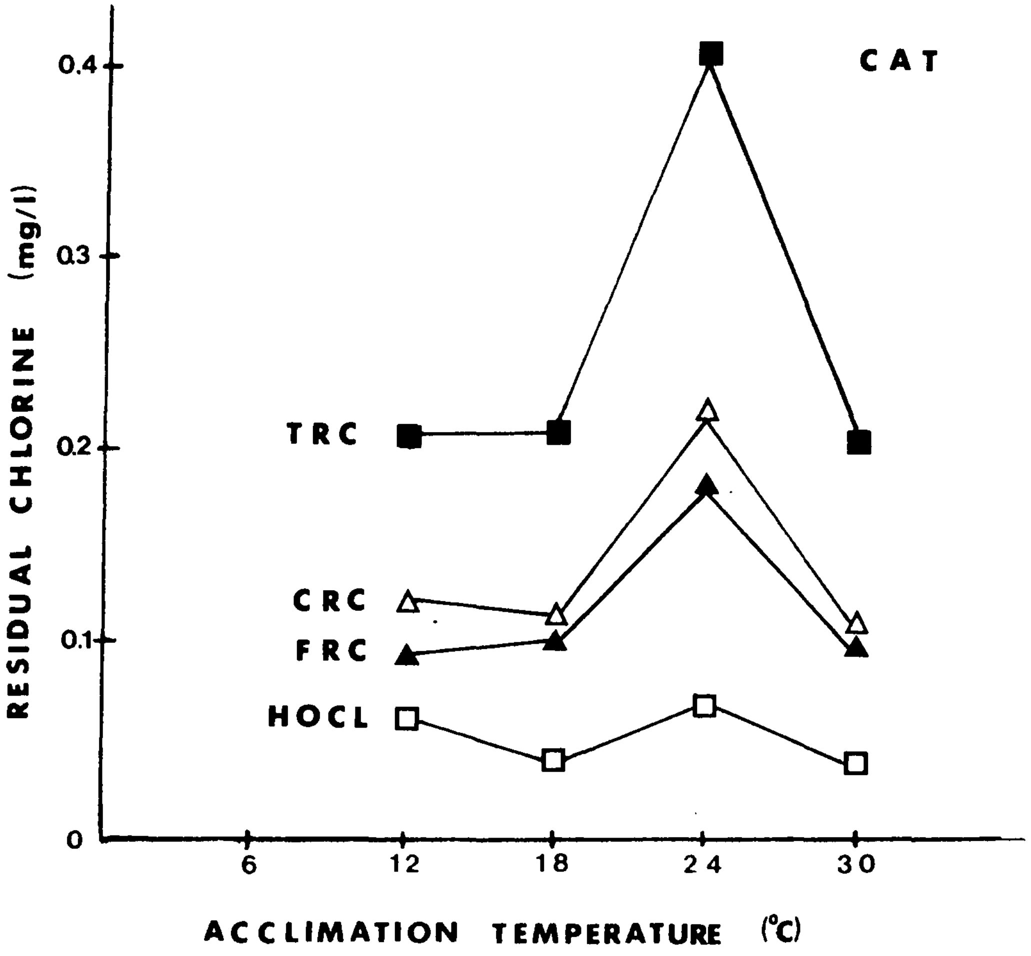

FIGURE 14 - Wilcoxon avoidance thresholds as a function of acclimation temperature for channel catfish.

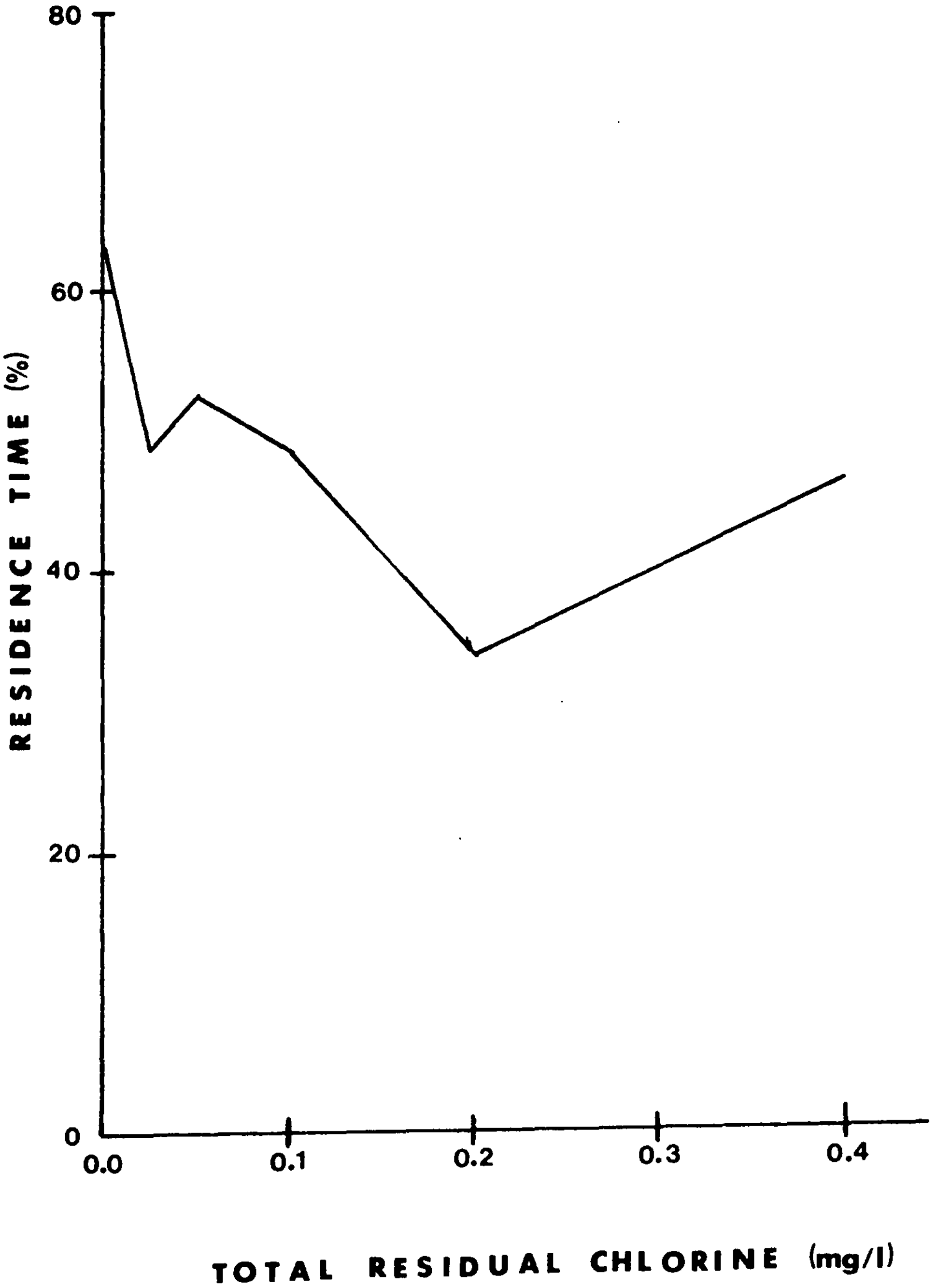

FIGURE 15 - Avoidance of TRC by carp acclimated at 6 C.

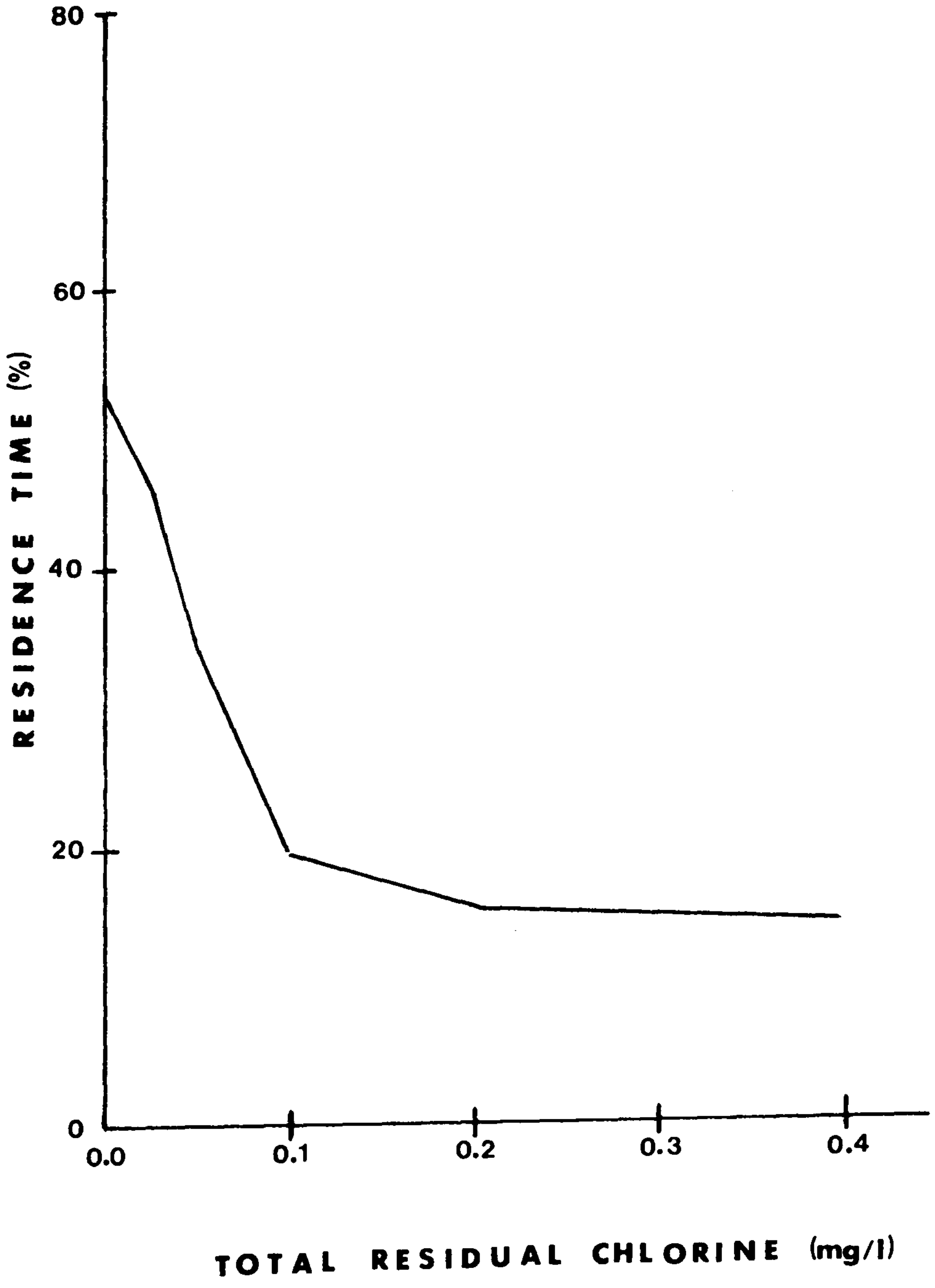

FIGURE 16 - Avoidance of TRC by carp acclimated at 12 C.

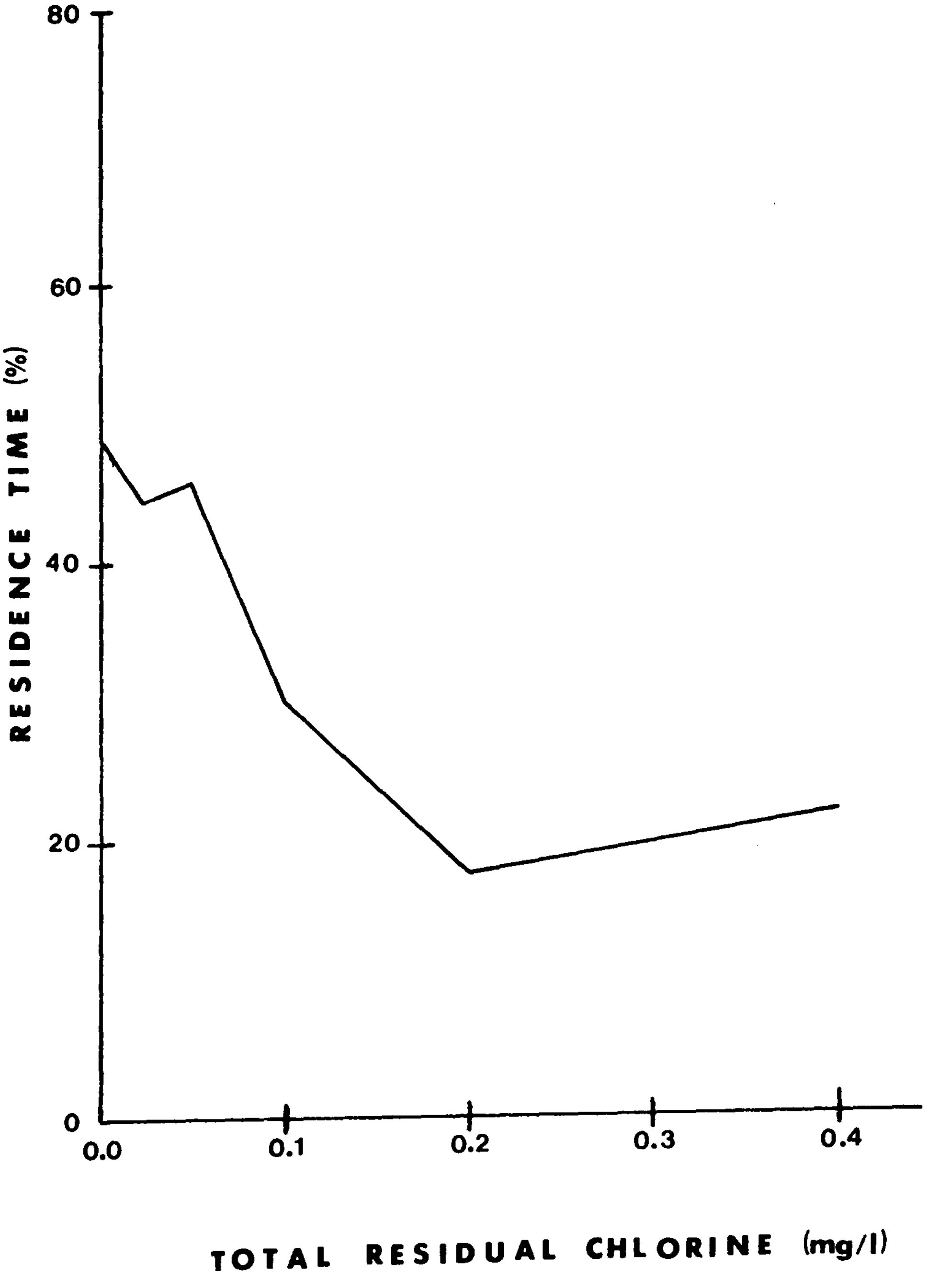

FIGURE 17 - Avoidance of TRC by carp acclimated at 18 C.

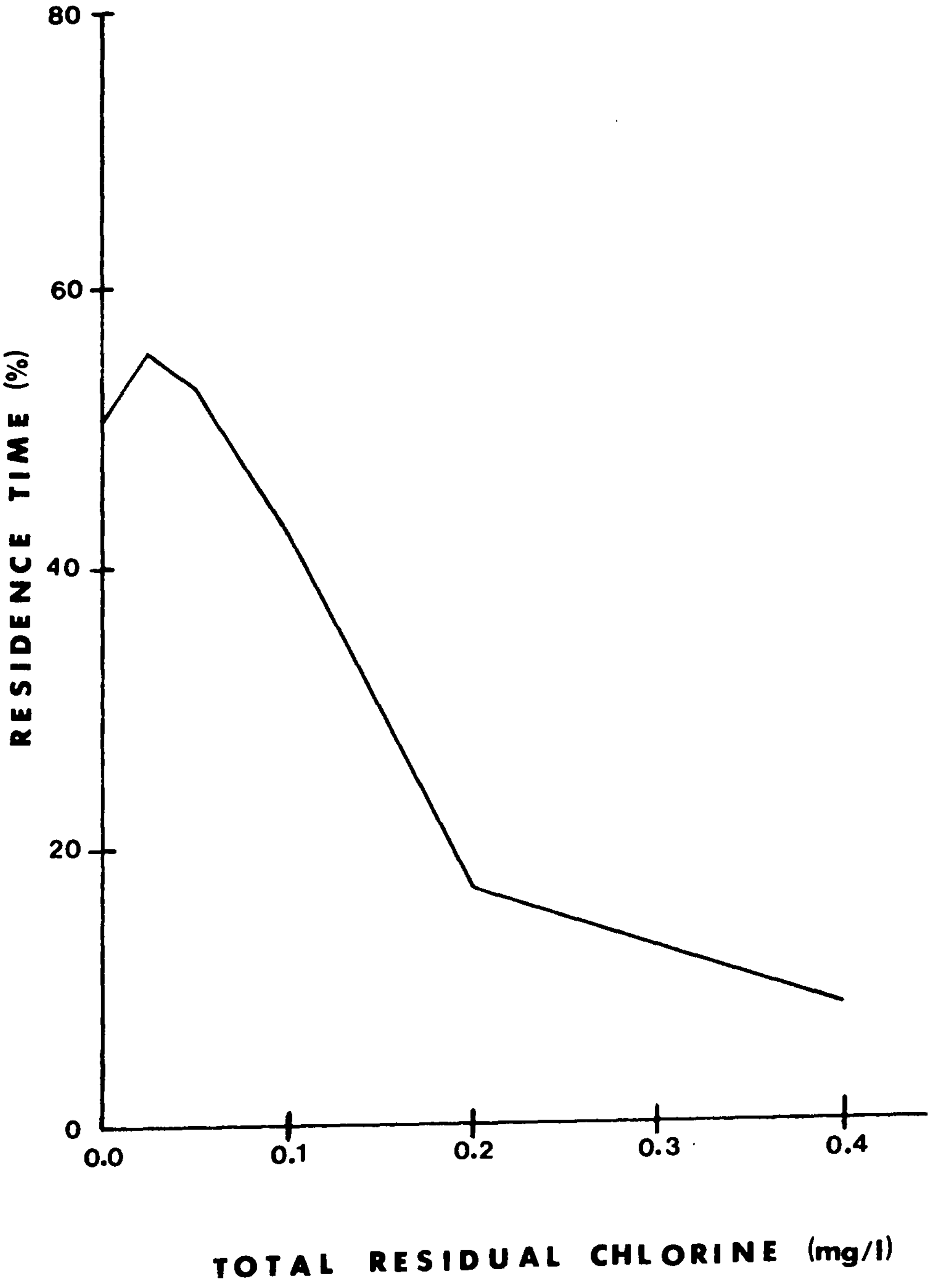

FIGURE 18 - Avoidance of TRC by carp acclimated at 24 C.

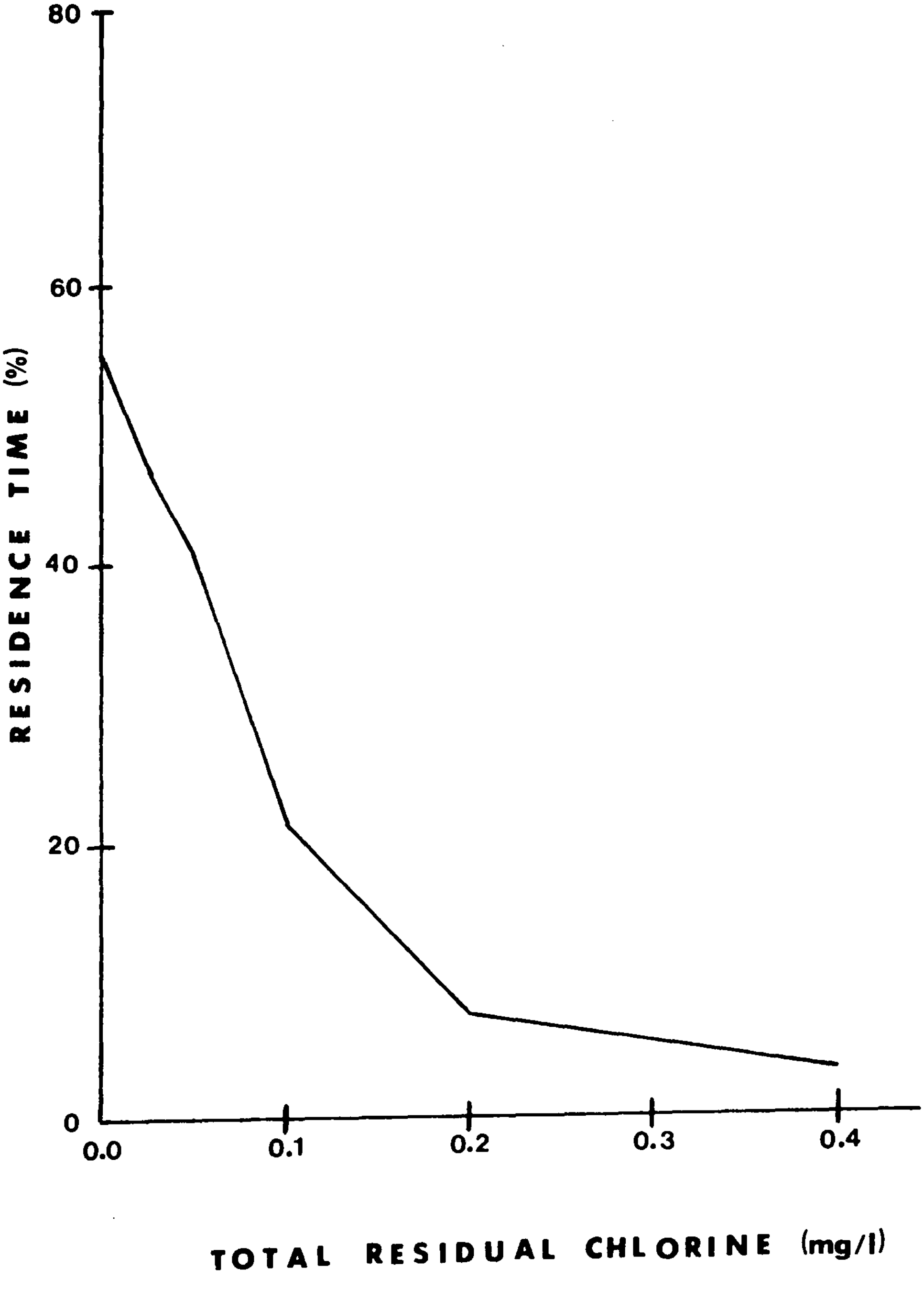

FIGURE 19 - Avoidance of TRC by carp acclimated at 30 C.

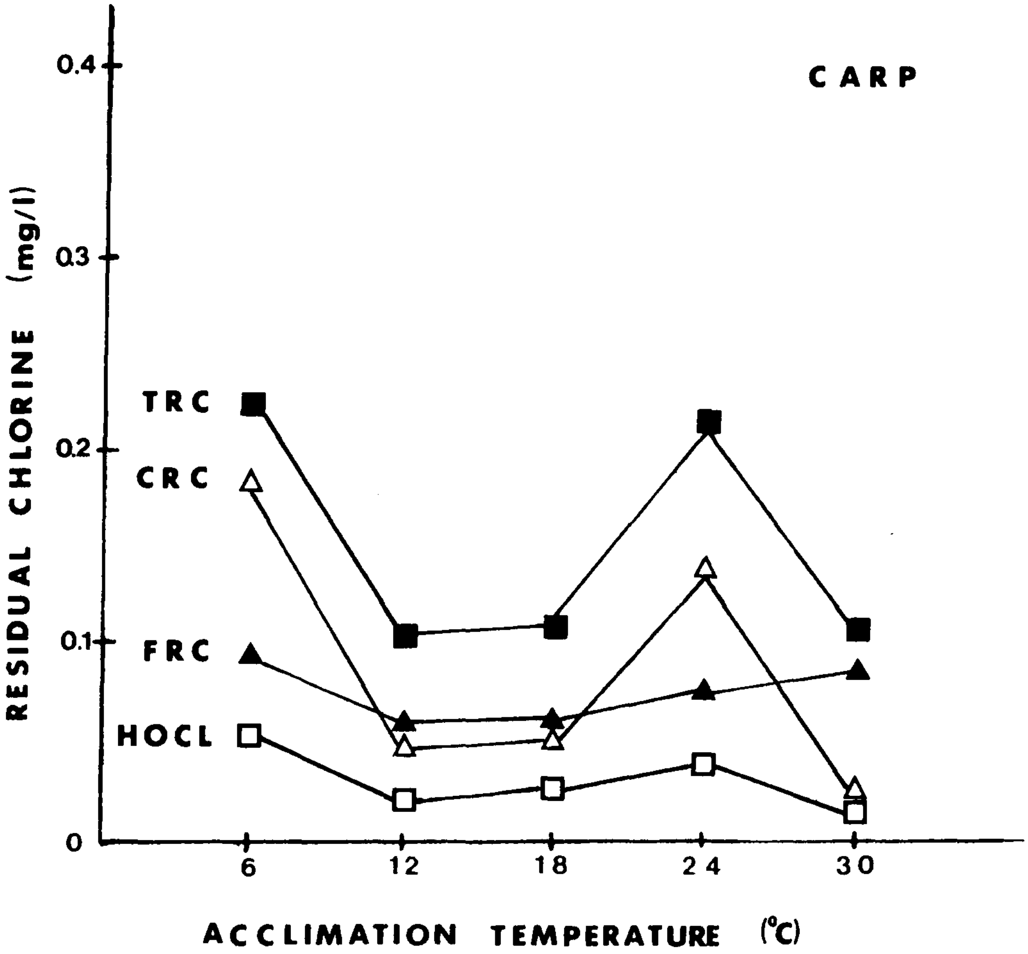

FIGURE 20 - Wilcoxon avoidance thresholds as a function acclimation temperature for carp.

The results of the Kruskal-Wallis Multiple Comparison Tests for the channel catfish, golden shiners and carp (FIGURES 21, 22, and 23 respectively) showed that the residual CRC and FRC concentrations at 12, 18, and 30 C had the same magnitude whereas the 24 C residual concentrations were slightly higher. The HOCl analyses for the carp and the golden shiners were practically identical. The minimum avoidance threshold for the carp was 0.014 mg/liter HOCl at 30 C and the maximum avoidance threshold was 0.017 mg/liter HOCl at 12, 18, and 24 C. For the golden shiners, the minimum avoidance threshold was 0.015 mg/liter HOCl at 30 C and the maximum avoidance threshold was 0.017 mg/liter HOCl at 24 C. The minimal variation in these data, among and between fish species and acclimation temperatures, strongly suggested that the hypochlorous acid residual most strongly directed fish avoidance behavior.

In the Kruskal-Wallis analysis for the channel catfish data, CRC and FRC avoidance thresholds varied with the acclimation temperature, but the HOCl avoidance thresholds were very consistent between temperatures (FIGURE 21). The primary difference was the fact that channel catfish avoided HOCl residuals four to five times as concentrated as did carp and golden shiners. The minimum and maximum avoidance thresholds occurred at 0.067 mg/liter at 24 C and 0.077 mg/liter HOCl at 30 C, respectively. The response to HOCl residuals was consistant between all acclimation temperatures, but the channel catfish appeared to be less sensitive to HOCl than

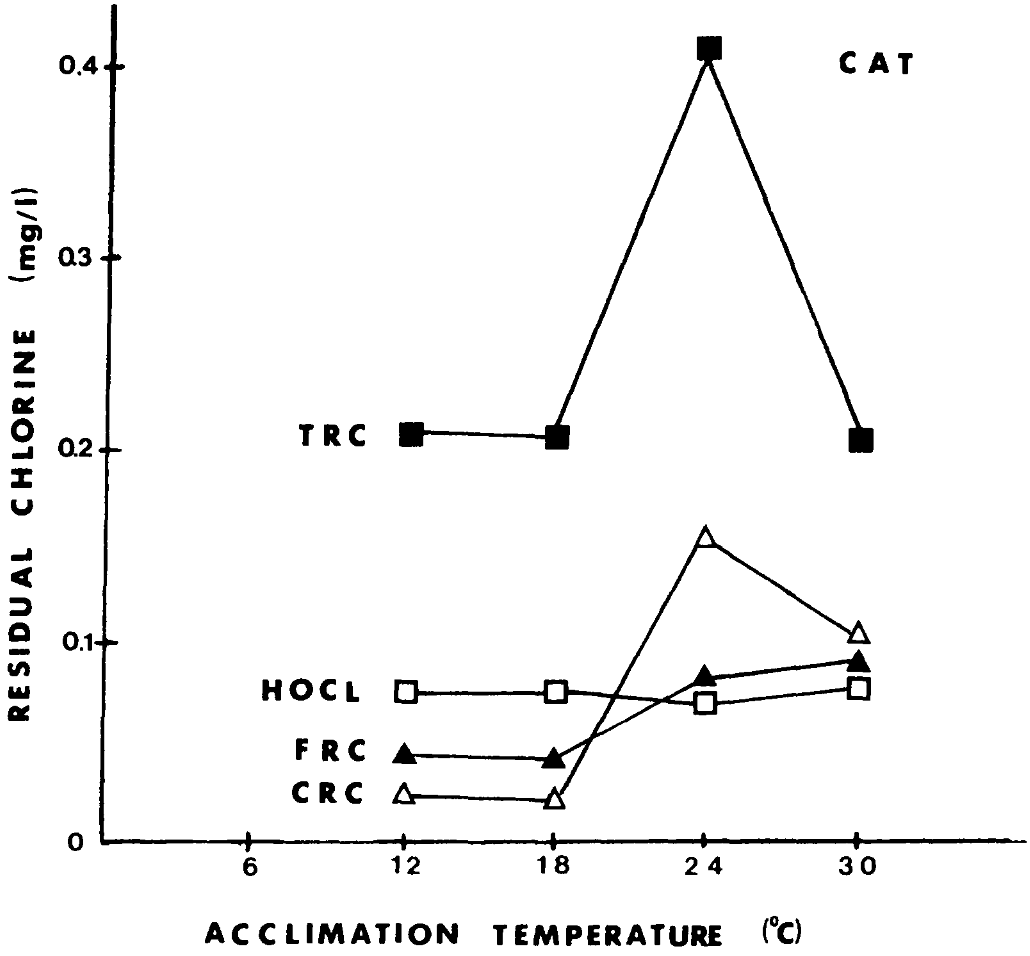

FIGURE 21 - Kruskal-Wallis avoidance thresholds as a function of acclimation temperature for channel catfish.

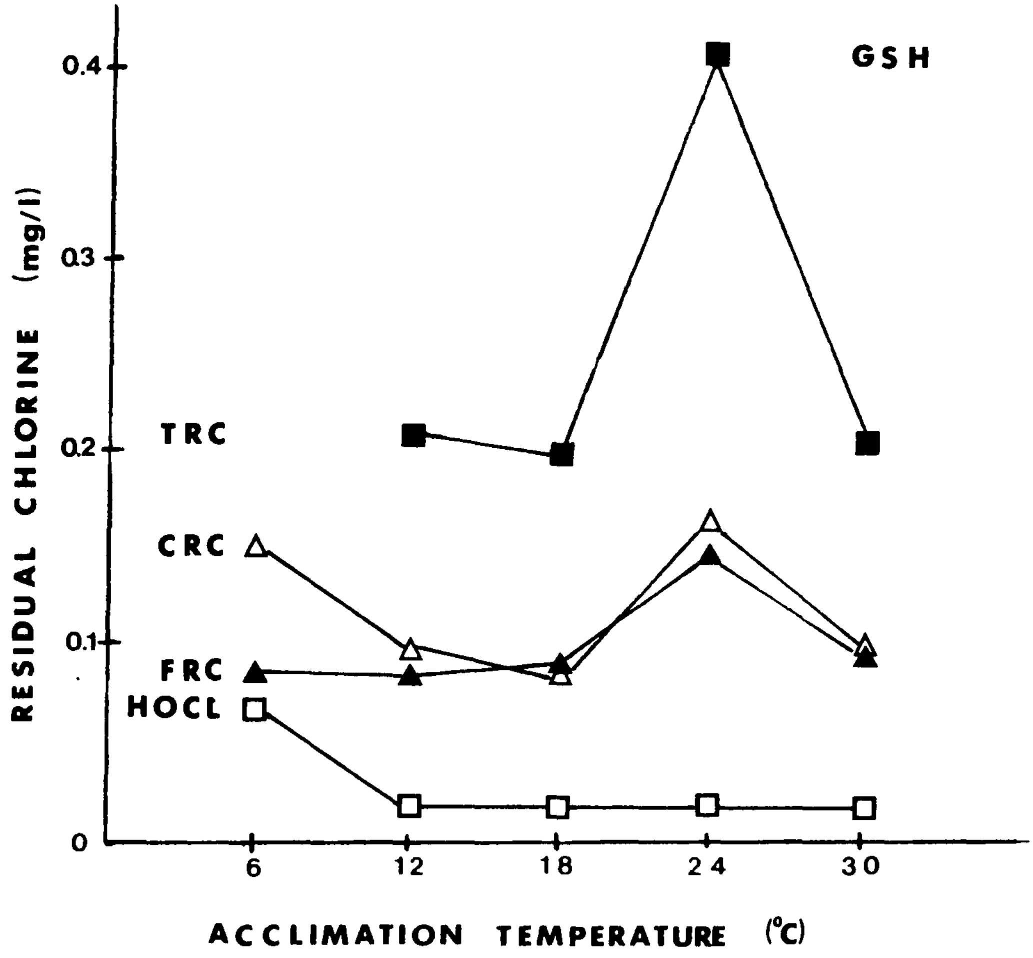

FIGURE 22 - Kruskal-Wallis avoidance thresholds as a function of acclimation temperature for golden shiners.

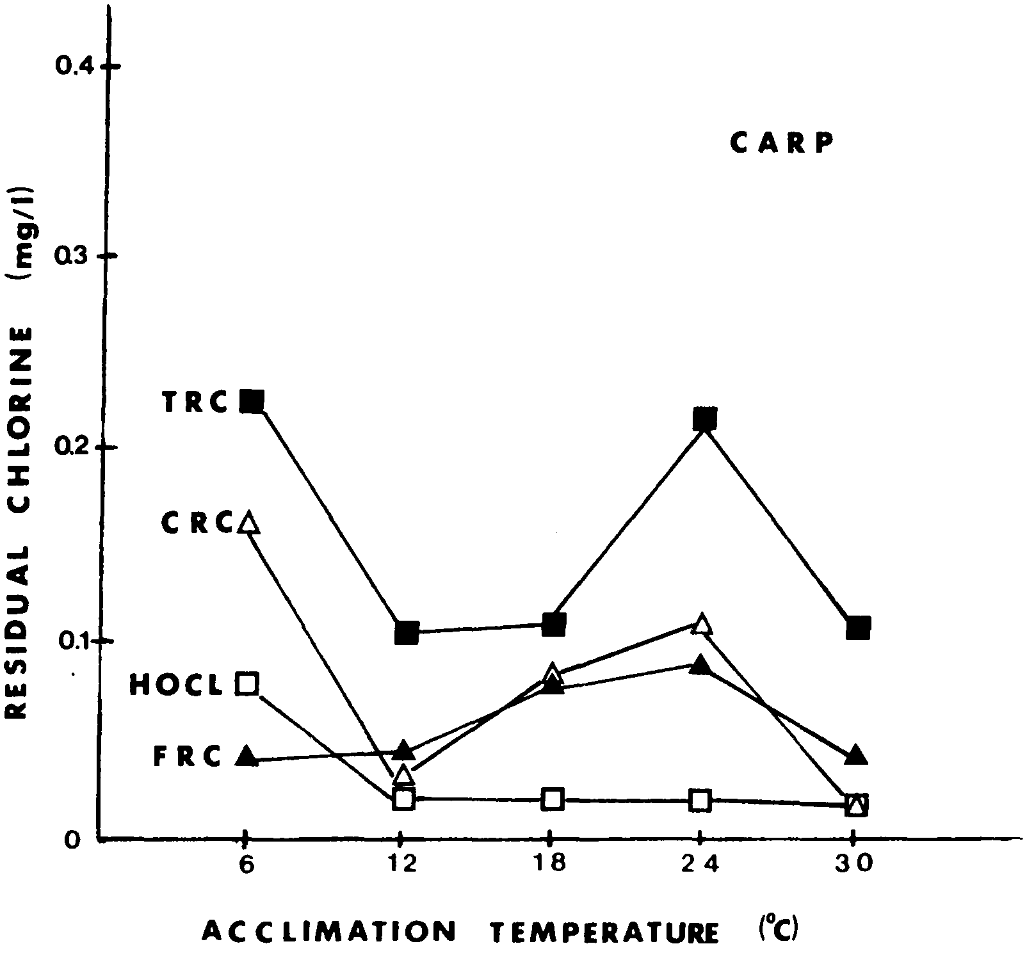

FIGURE 23 - Kruskal-Wallis avoidance thresholds as a function of acclimation temperature for carp.

were the carp and golden shiner.

## Temperature Preference Trials

Temperature preferenda varied with the species of fish and acclimation temperature tested (TABLE 3). The carp preferred 24 C at 12-C acclimation temperature and 29 C at 24-C acclimation temperature. These means agree favorably with Pitt *et al.* (1956) who found that carp acclimated at 15 and 25 C preferred 25 and 31 C respectively. All preferenda are well below reported upper lethal temperatures of 31-34-C (Black, 1953). The golden shiner preferred 20 C at 12-C acclimation temperatures and 27 C at 24-C acclimation temperatures. These preferenda were also well below upper lethal temperatures (Alpaugh, 1972). The channel catfish preferred 20 C at 12-C acclimation temperatures and 29 C at 24-C acclimation temperatures.

## Combined Temperature Preference/Chlorine Avoidance Experiments

HOCl avoidance thresholds for chlorinated waters heated to the preferred temperature were much greater than the avoidance thresholds for chlorinated waters without elevated temperatures for the carp and golden shiner. At 12 C, the golden shiner significantly ($P=.05$ level) preferred heated water at sublethal chlorine doses (0.025 and 0.050 mg/l TRC). The avoidance threshold of 0.192 mg/liter TRC was nearly identical to the conventional avoidance threshold of 0.209 mg/liter TRC (FIGURE 24, Appendix). Carp and

TABLE 3 - Temperature preferenda derived from shallow horizontal temperature gradients.

| Species | Acclimation Temperature | Selected Temperature |
|---|---|---|
| *Cyprinus carpio* | 12 C | 24 C |
| (carp) | 24 C | 29 C |
| *Ictalurus punctatus* | 12 C | 20 C |
| (channel catfish) | 24 C | 29 C |
| *Notemigonus crysoleucas* | 12 C | 20 C |
| (golden shiner) | 24 C | 27 C |

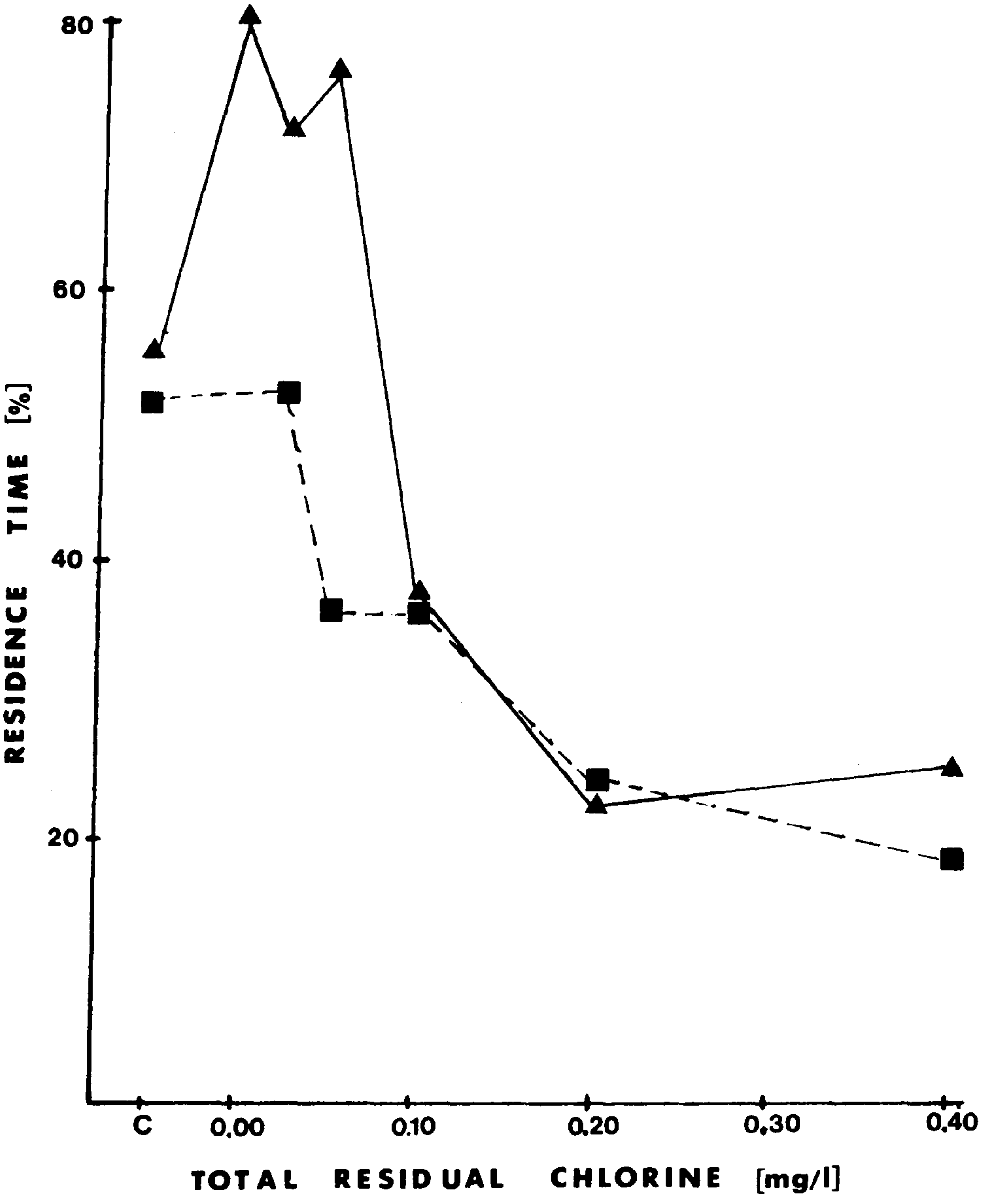

FIGURE 24 - Avoidance response by golden shiners to heated ( ▲ ) and unheated ( ■ ) chlorine gradients when tested at 12 C.

channel catfish behavior was erratic at the 12-C acclimation temperature (Appendix). Carp did not avoid the water at any TRC concentration and the residence time was above 50% at every treatment (FIGURE 25). The channel catfish response was completely different in that treated water repelled the fish at every TRC concentration (FIGURE 26). The avoidance threshold occurred at 0.025 mg/liter TRC.

The response of fish at the 24-C acclimation temperature was more precise than their behavior at 12 C (FIGURES 27-29, Appendix). For all three species, percent residence times were significantly higher than the control data at treatments of 0.000 and 0.025 mg/liter TRC, thus confirming their preference for warmer water. At concentrations of 0.050 mg/liter and higher, percent residence times decreased as the target TRC concentrations were increased. The avoidance thresholds for the golden shiner, carp and channel catfish were 0.217, 0.205, and 0.107 mg/liter TRC, respectively.

The Kruskal-Wallis avoidance thresholds for the golden shiner at 12 C were 0.192 mg/liter TRC, 0.085 mg/liter CRC, 0.101 mg/liter FRC and 0.065 mg/liter HOCl. The channel catfish avoided every treatment and the carp showed no avoidance at 12 C. At 24-C acclimation temperature, the TRC, CRC and FRC avoidance thresholds varied from species to species (TABLE 4). Hypochlorous acid avoidance thresholds were nearly identical for each species: 0.074 mg/liter for the carp, 0.077 mg/liter for the golden shiner and 0.079 mg/liter for the channel catfish. This precision implies once again that the

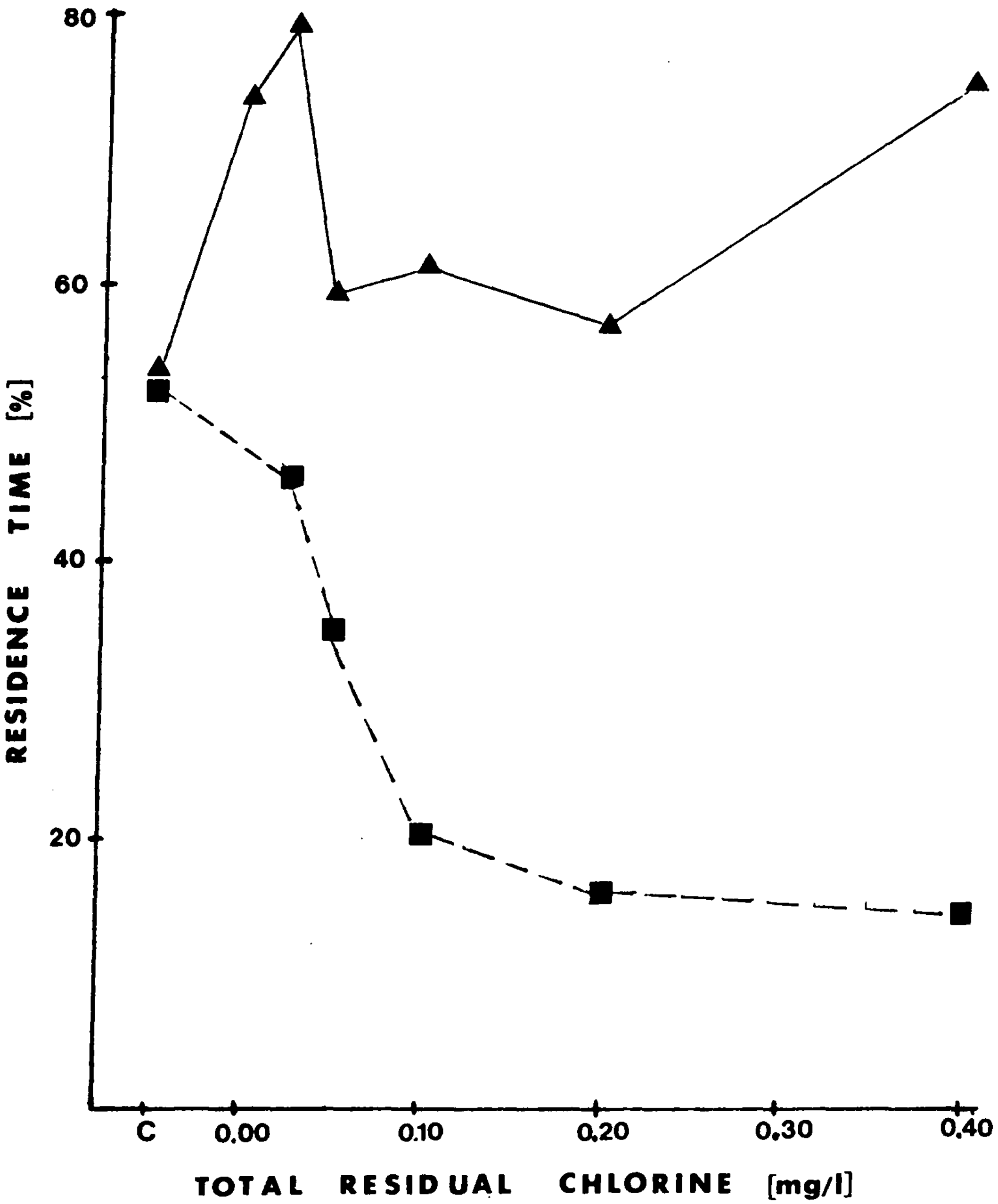

FIGURE 25 - Avoidance response by carp to heated ( ▲ ) and unheated ( ■ ) chlorine gradients when tested at 12 C.

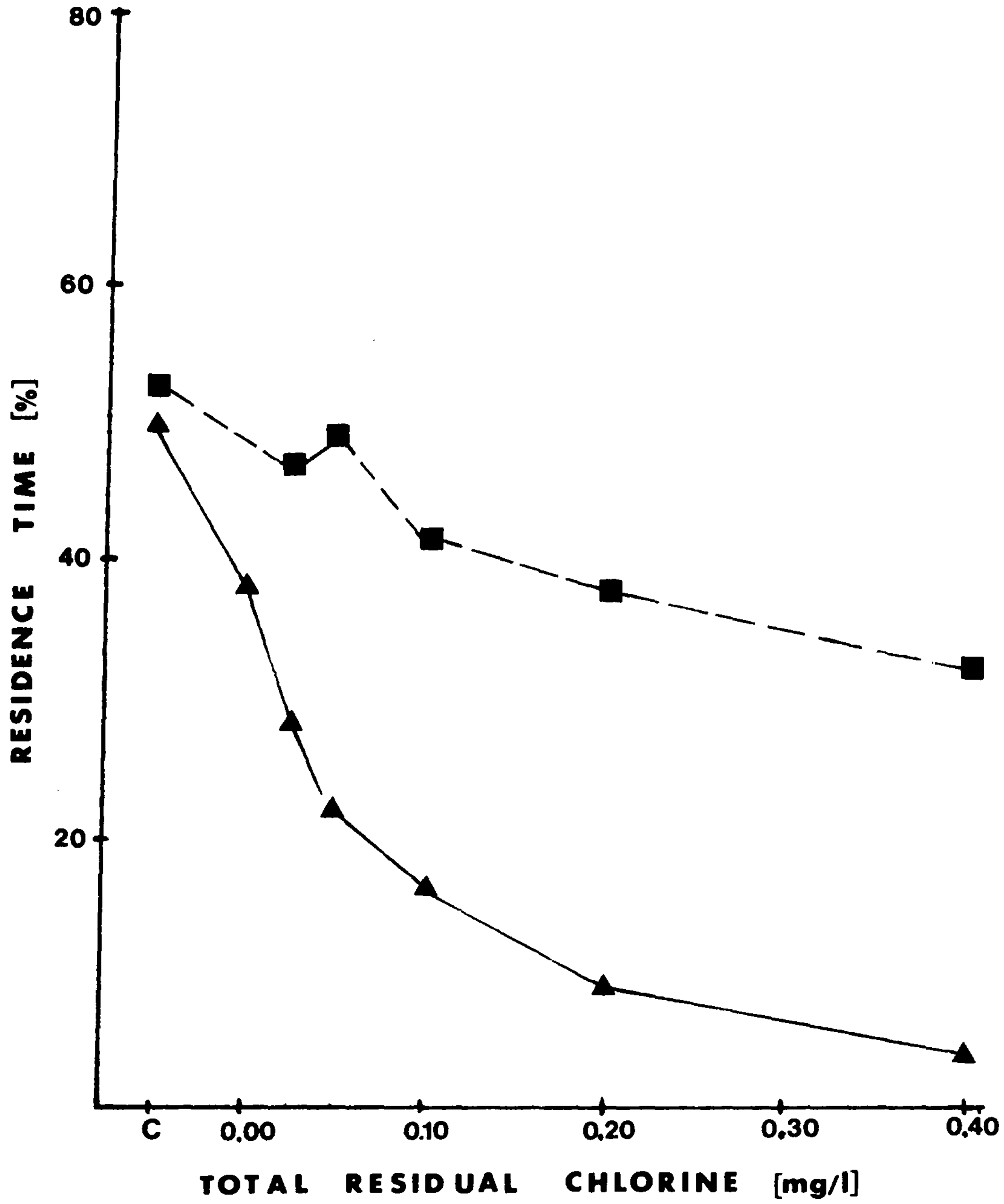

FIGURE 26 - Avoidance response by channel catfish to heated ( ▲ ) and unheated ( ■ ) chlorine gradients when tested at 12 C.

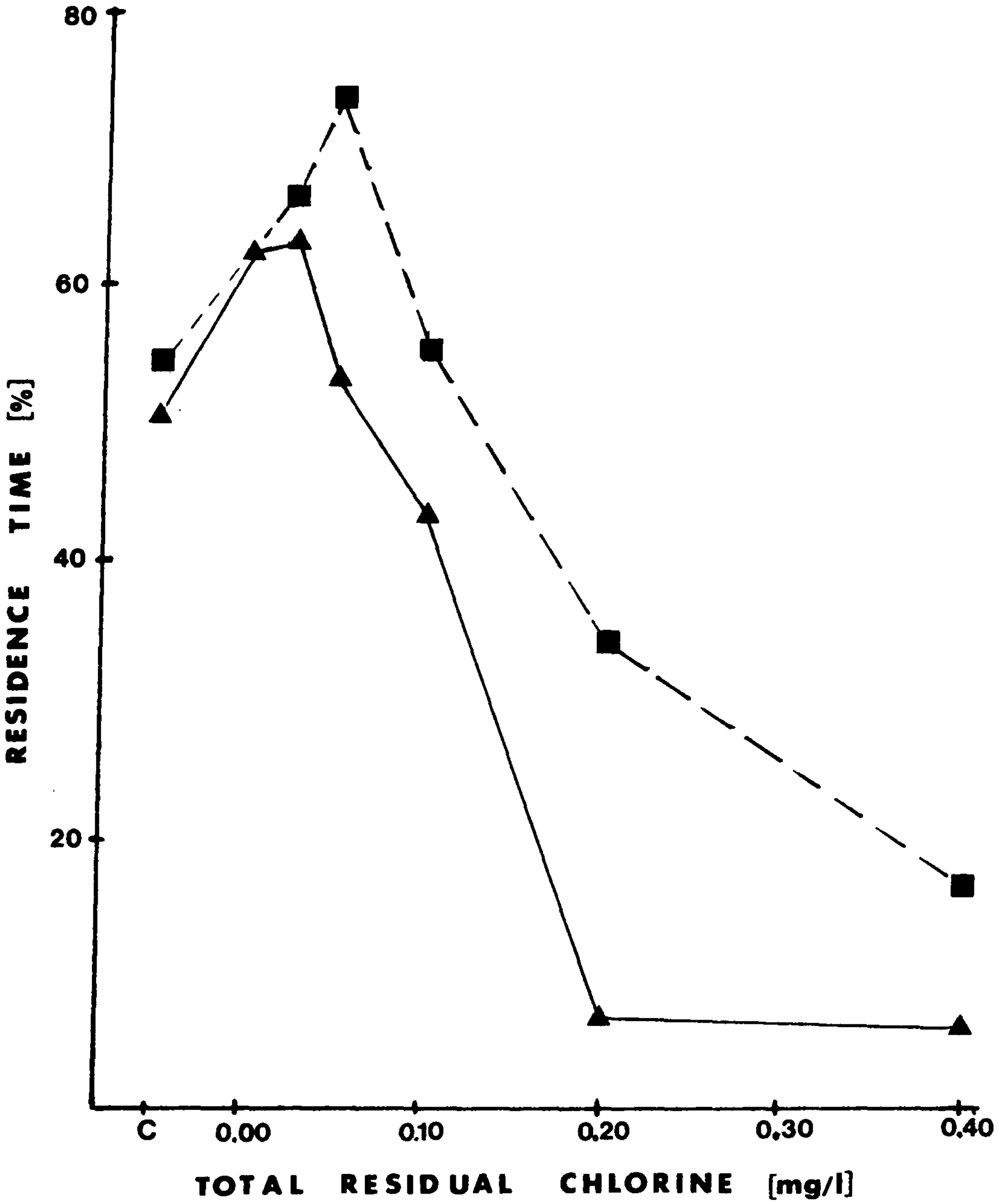

FIGURE 27 - Avoidance response by golden shiner to heated ( ▲ ) and unheated ( ■ ) chlorine gradients when tested at 24 C.

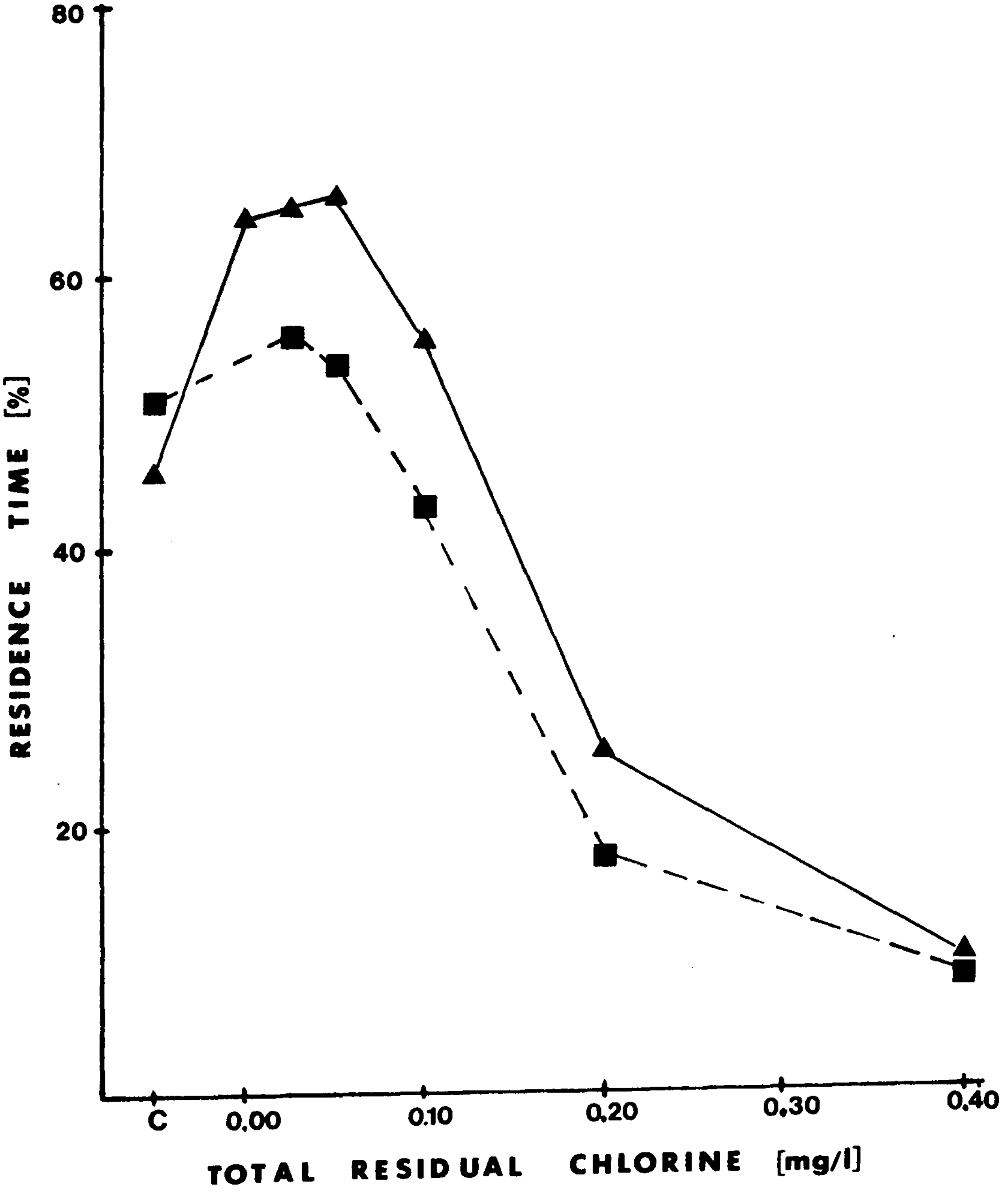

FIGURE 28 - Avoidance response by carp to heated ( ▲ ) and unheated ( ■ ) chlorine gradients when tested at 24 C.

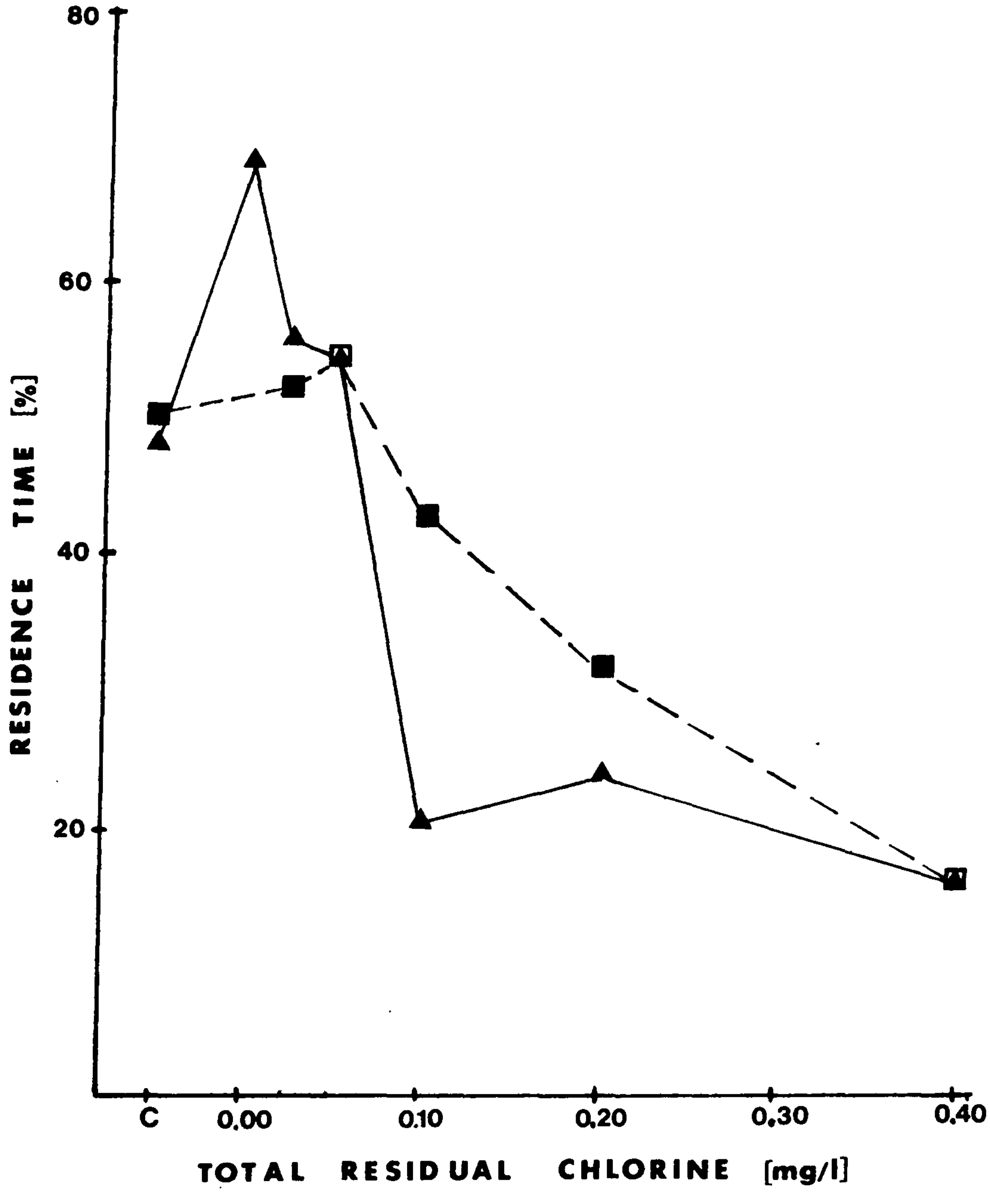

FIGURE 29 - Avoidance response by channel catfish to heated ( ▲ ) and unheated ( ■ ) chlorine gradients when tested at 24 C.

TABLE 4 - Combined Temperature preference/Chlorine avoidance thresholds derived from Kruskal-Wallis Multiple Comparison Tests ( $\bar{X} \pm$ S.E. ).

| Threshold Residual (mg/l) | Carp | Channel Catfish | Golden Shiners |
|---|---|---|---|
| | 12-C Acclimation | Temperature | |
| TRC | - | 0 | 0.192±0.003 |
| CRC | - | 0 | 0.085±0.005 |
| FRC | - | 0 | 0.101±0.004 |
| HOCl | - | 0 | 0.065±0.005 |
| | 24-C Acclimation | Temperature | |
| TRC | 0.205±0.004 | 0.107±0.002 | 0.217±0.012 |
| CRC | 0.141±0.003 | 0.088±0.005 | 0.087±0.004 |
| FRC | 0.091±0.007 | 0.086±0.007 | 0.102±0.003 |
| HOCL | 0.074±0.004 | 0.079±0.004 | 0.077±0.005 |

fish responsed primarily to changes in HOCl in test solutions and not specifically to changes in the TRC, CRC, or FRC.

## AVOIDANCE INDICES

Neither the mean time per entry or the number of entries into the treated water were adequate indices of avoidance behavior. The number of entries dropped from 15 at 0.100 mg/liter TRC to 7 entries at 0.200 mg/liter TRC, but increased again to 15 at 0.400 mg/liter TRC (FIGURE 30). On the other hand, the average time of each entry steadily decreased as the chlorine concentration was increased (FIGURE 31). The drop was not significant because the data at any one chlorine concentration was extremely variable. For example, the average time of each entry at 0.000 mg/liter TRC was 22 seconds but the minimum value was 13 seconds and the maximum value was 47 seconds. Both of these indices consumed much more time and effort to collect than did the elapsed residence time. After considering their low sensitivity, these indices were not recommended as adequate measures of avoidance behavior.

## EXPOSURES TO SINGLE CHLORINE DOSES

The absence of a recent exposure to low chlorine concentrations did not modify the behavior of fish at higher chlorine concentrations. The comparison of regular chlorine avoidance data and the supplementary experiments were very similar (FIGURE 32). The single-dose avoidance threshold occurred at 0.208 mg/liter TRC with

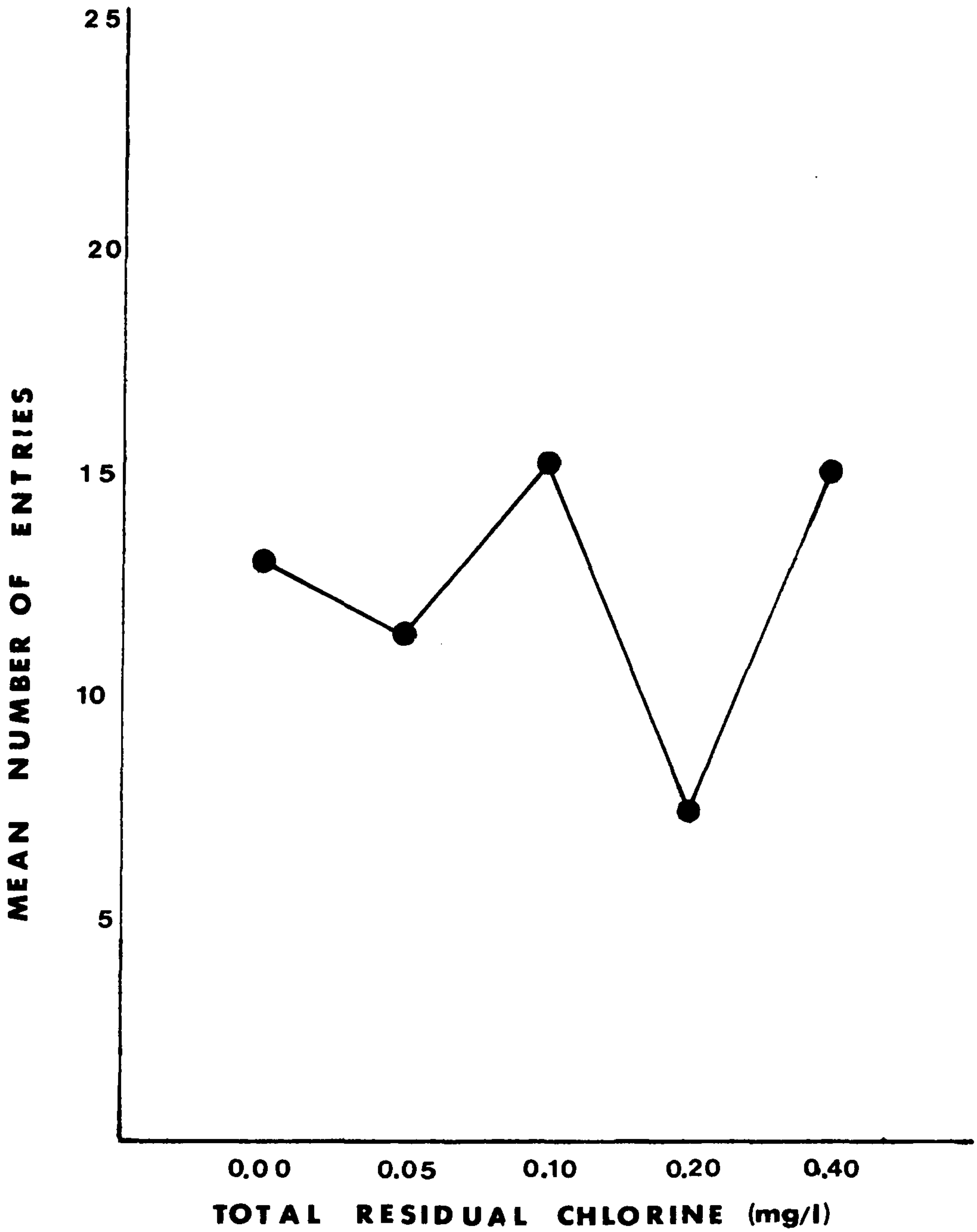

FIGURE 30 - Number of entries by golden shiners at 18 C into the treated water at various target TRC.

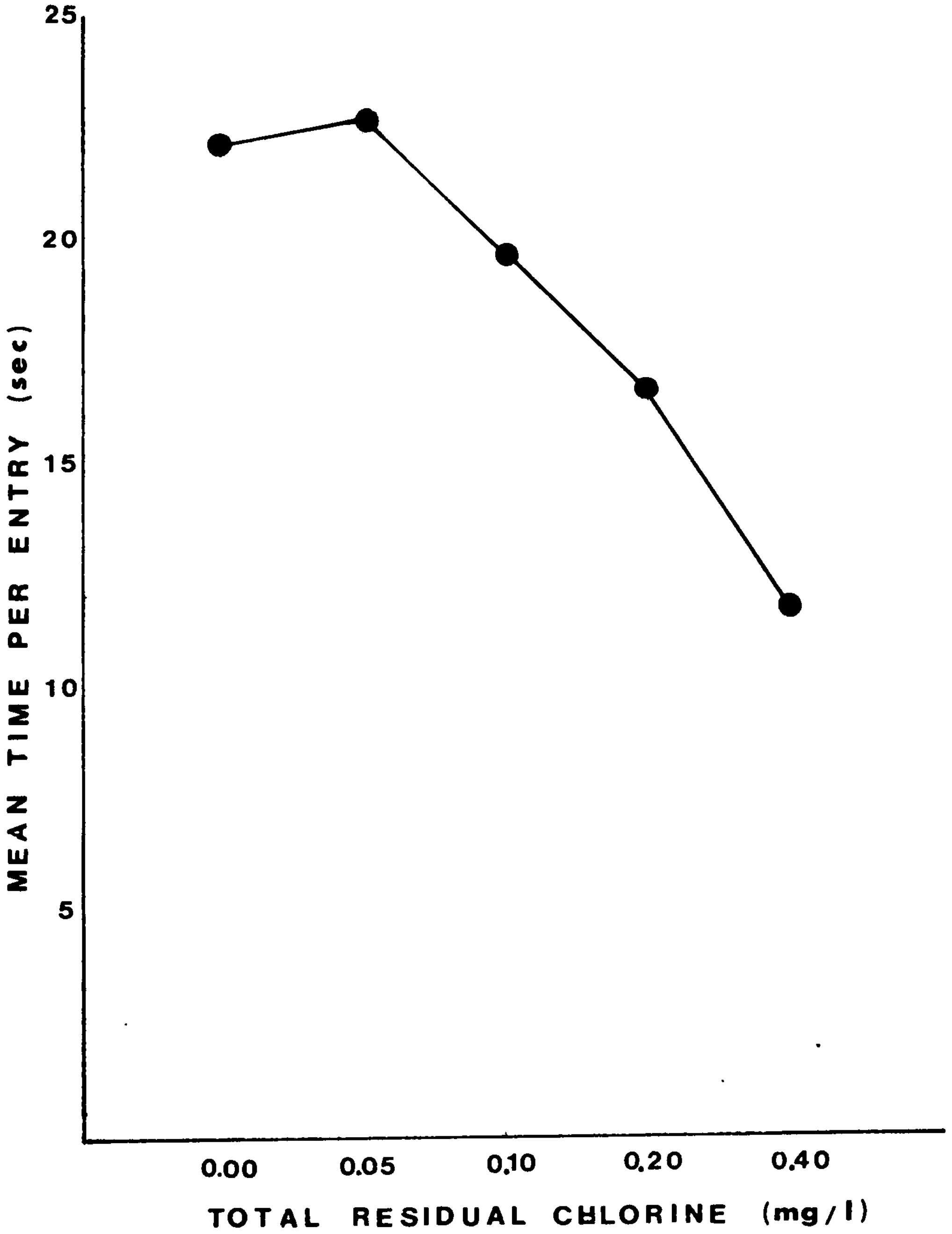

FIGURE 31 - Mean time per entry by golden shiners at 18 C into treated water at various target TRC.

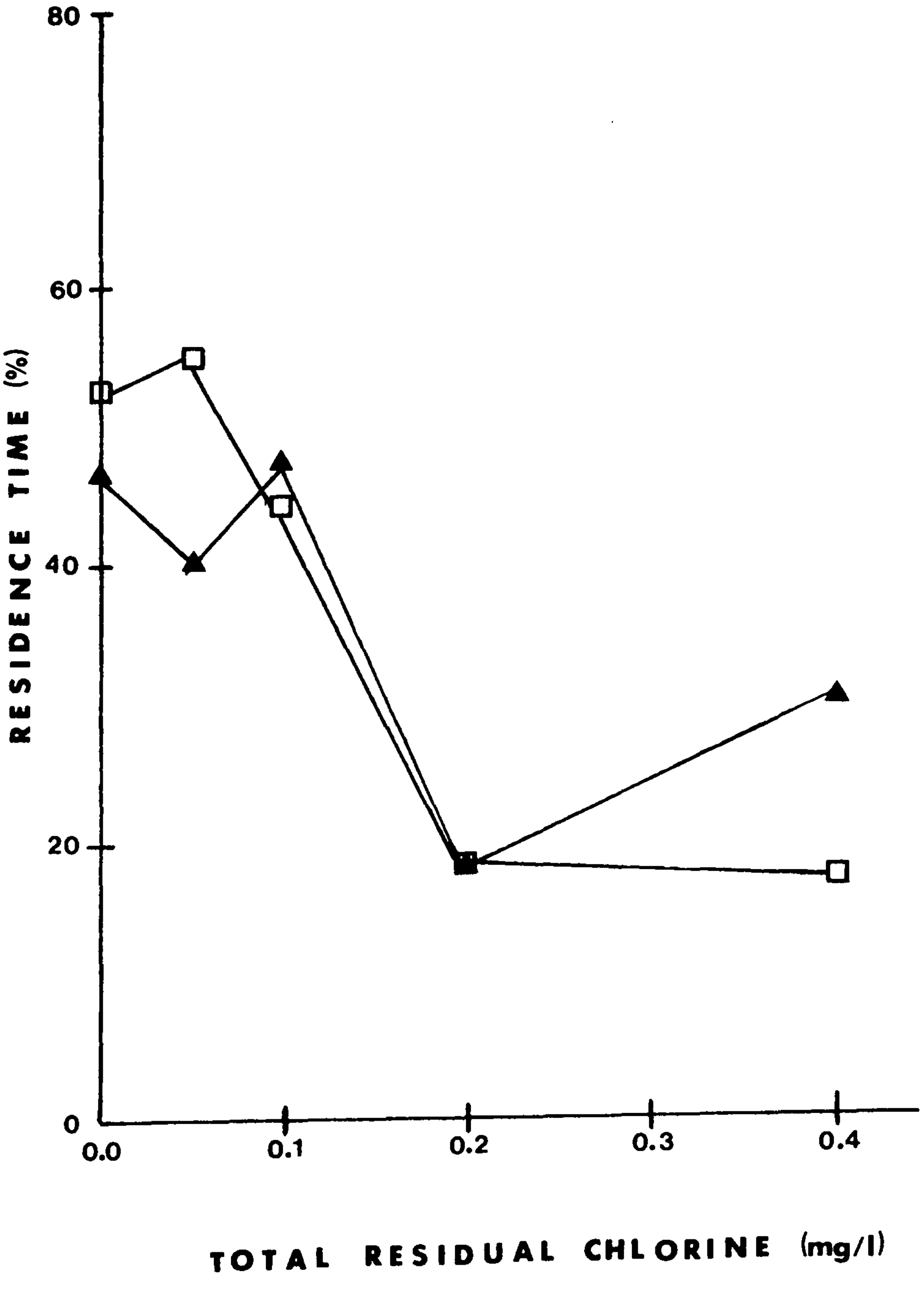

FIGURE 32 - Avoidance of single TRC doses ( ▲ ) and multiple TRC doses ( □ ) by golden shiners at 18 C.

residual concentrations of 0.112 mg/liter CRC, 0.096 mg/liter FRC and 0.052 mg/liter HOCl. The regular avoidance thresholds were 0.199 mg/liter TRC with residual concentrations of 0.112 mg/liter CRC, 0.086 mg/liter FRC and 0.027 mg/liter HOCl. The results indicated that possible physiological perturbations of the fish did not result from previous exposures to low chlorine concentrations, and the fish did not learn to avoid certain regions of the trough from previous exposures.

# V. DISCUSSION

## THE EFFECT OF ACCLIMATION TEMPERATURE ON CHLORINE AVOIDANCE

Acclimation temperatures greatly affect the general activity of fish by directly accelerating basic chemical processes within each cell. Key enzymatic reactions and metabolic processes are directly dependent on the ambient temperature, because fish are strict poikilotherms. These effects translate into depressed activity at lower temperatures and increased activity at higher temperatures. These extreme situations were evident at the 6 and 30-C acclimation temperatures. At 30 C, fish generally moved very quickly, but did not appear to be unduly stressed. Seven day lethal upper temperature limits were usually several degrees higher than this acclimation temperature. On the other hand, fish acclimated at 6 C were quite sluggish but there was no visible evidence of stress.

Although acclimation temperature clearly influences the general activity of each fish, it had no effect on the HOCl avoidance thresholds at 12, 18, 24 and 30 C. The HOCl concentrations were relatively constant at all acclimation temperatures. The TRC, CRC, and FRC avoidance thresholds were not consistent, but varied greatly with the quality of the New River water. Clearly, the effect of temperature on the HOCl dissociation constant (Equation 4) affects avoidance behavior more than its effect on the rate of chemical reactions within the fish.

Sprague and Drury (1969) found that rainbow trout slightly avoided 0.001 mg/liter TRC and strongly avoided 1.000 mg/liter TRC. The same species significantly preferred 0.100 mg/liter TRC, a level lethal in four days. Bogardus *et al*. (unpublished manuscript) determined that *Notropis volucellus*, *Notropis blennius* and *Pimephales vigilax* avoided 0.005, 0.150, and 0.050 mg/liter TRC, respectively. Fava and Tsai (1976) found that blacknose dace (*Rhinichthys atratulus*) avoided chloramine solutions of 0.051 mg/liter and free residual chlorine solutions of 0.920 mg/liter. In the present study, steep toxic gradients revealed that Wilcoxon chlorine avoidance thresholds varied from 0.104 to 0.403 mg/liter TRC, 0.056 to 0.255 mg/liter CRC, 0.049 to 0.219 mg/liter FRC and 0.019 to 0.066 mg/liter HOCl. Preference for the treated water was frequently observed at low chlorine concentrations (0.025 or 0.050 mg/liter TRC), but these concentrations were well above lethal levels.

Practically every investigator employed a different avoidance trough (creating steep gradients, shallow gradients, etc.) and/or different indices of avoidance behavior (residence time, number of entries, etc.). For this reason, comparisons between studies cannot be accurately made, emphasizing the need for more standardized materials and methods.

## EFFECT OF STEEP THERMAL GRADIENTS ON CHLORINE AVOIDANCE THRESHOLDS

The warmer, preference temperature water in the experimental troughs normally attracted the fish tested. When the fish crossed the central drains, it was obvious that they sensed the different temperatures, because they often circled at the interface before re-entering the heated water. This selection process was presumably performed with the aid of subcutaneous receptors which set up nerve impulses that eventually lead to overt behavioral responses (Sullivan, 1954). Therefore, thermal gradients are directive factors since they help the organism orient within its environment (Fry, 1947). Acclimation temperatures cannot function as directive factors because they do not guide the fish in its environment. Detailed reviews of temperature as a directive factor are presented by Brett (1956) and Ferguson (1958).

The orientation of an animal is determined by many factors. For any organism, these factors are hierarchically arranged, with some factors exerting a greater influence on orientation than do others. Temperature and chlorine are only two factors involved in fish orientation about power plant discharges.

The HOCl avoidance thresholds obtained from temperature preference/chlorine interactions for the carp and golden shiners at 24 C were 5-6 times as great as the conventional chlorine avoidance thresholds, where the temperature is constant throughout the trough. These higher concentrations suggest that chlorine becomes less toxic at higher temperatures. This hypothesis is unlikely because Stober and Hanson (1974) found that total residual chlorine was more toxic to salmonid fish at higher temperatures and Capuzzo _et al._ (1976) found that free residual chlorine was more toxic to lobsters at higher temperatures. A more probable hypothesis is that temperature responses are higher in the behavioral hierarchy than are the responses to weak chlorine solutions. In this case, the attraction of the warmer water over-rides the repulsion by the hypochlorous acid at low chlorine concentrations. The chlorine eventually becomes so concentrated that the general irritation it causes the fish (Hiatt, 1953) exceeds the attraction of the warmer water. The chlorine does not become less toxic but the avoidance threshold is raised by the warmer water.

The channel catfish thresholds are about equal in the combined temperature preference/chlorine avoidance experiments. Steep thermal gradients do not modify their behavior. Apparently, the behavioral hierarchy is reversed with the chlorine response dominant over the temperature response.

## Lethal Levels And Avoidance Thresholds

The data from the present study can be compared to few other studies. Acute bioassays have been performed by Panikkar (1960) and Esvelt _et al._ (1971) but they only reported TRC values. Chronic bioassays have mainly examined chloramine toxicity (Arthur and Eaton, 1971).

Heath (in press) conducted chlorine bioassays directly applicable to power plant effluents which are relevant to this study. Several fish species, including the golden shiner, carp, and channel catfish, were subjected intermittently to three, 45-min chlorine pulses a day. Median lethal concentration and median lethal time at several chlorine concentrations were determined at low (5 or 6 C) and high (24 C) acclimation temperatures. The pH was determined and the TRC and FRC were measured amperometrically.

The 96 hour median lethal CRC, FRC and HOCl concentrations were lower for all species tested at 24 C than at 5 or 6 C (TABLE 5). This suggested that chlorine became more toxic at higher temperatures as hypothesized by Cairns _et_ _al._ (1975). The increased toxicity at higher temperatures is explained by chlorine's mode of action. Chloramines are believed to enter the fish's blood directly and oxidize up to 40% of the hemoglobin to methemoglobin (Grothe and Eaton, 1975). Methemoglobin cannot act reversibly as an oxygen carrier, and a decrease in $O_2$ concentration ultimately results. Free residual chlorine stimulates mucous formation at the gill surfaces and does not significantly alter methemoglobin concentrations. However, it does cause a severe drop in arterial $pO_2$ (Bass and Heath, in press).

The end result of the action of both chlorine residuals is asphyxiation. Any factor which reduces oxygen availability also reduces the affected organism's chance for survival. If all other

TABLE 5 - 96 Hour median lethal concentrations (mg/liter) for intermittent chlorination. All concentrations were measured at the peak of each chlorine pulse (from Heath, In press).

| Species | Acclimation Temperature | CRC | FRC | HOCl |
|---|---|---|---|---|
| Carp | 6 | 1.720 | 0.538 | 0.354 |
| | 24 | - | 0.219 | 0.116 |
| Channel Catfish | 5 | 0.275 | 0.082 | 0.048 |
| | 24 | 0.260 | 0.064 | 0.034 |
| Golden Shiner | 5 | 0.724 | 0.269 | 0.157 |
| | 24 | 0.930 | 0.193 | 0.102 |

factors are equal, warm-chlorinated water would be more toxic to stressed fish because it holds less dissolved oxygen than does cold chlorinated water. On the other hand, all factors are not equal and cold chlorinated waters may be more toxic because the ratio of HOCl/FRC is higher than it is in warmer water (Equation 4). As a result, the increased toxicity due to the higher HOCl concentrations is offset somewhat by the increased dissolved oxygen. Therefore, it is difficult to make any generalizations concerning the toxicity of cold versus warm-chlorinated waters.

Median lethal times at various FRC concentrations were provided by Heath (in press). Temperature and pH were also reported and I was able to calculate HOCl concentrations (Equations 4 and 5). The logarithm of the time was then plotted against the logarithm of the HOCl concentration and a "best fit" line derived by linear regression (Appendix). At any chlorine concentration, the median survival time could then be approximated (TABLE 6).

The behavior of the carp and golden shiner to hypochlorous acid was very similar. Both species were very sensitive to chlorine and avoided 1/6 of the 96 hour median lethal HOCl concentration. Even if the golden shiner were trapped in some way and could not avoid the discharge area, they could presumably survive for 2141 hours. $LT_{50}$ values were not reported for the carp at 24 C but because of their behavior's similarity to that of the golden shiner in all other trials, it is assumed that their survival would be similar.

TABLE 6 - Median lethal times (hrs) for hypochlorous acid avoidance thresholds (mg/liter) for fish acclimated at 24 C. All concentrations were measured at the peak of each chlorine pulse (from Heath, In press).

| Species | Chlorine Avoidance Threshold | $LT_{50}$ | Temp/chlorine Avoidance Threshold | $LT_{50}$ |
|---|---|---|---|---|
| Carp | 0.017 | - | 0.074 | - |
| Channel Catfish | 0.067 | 38 | 0.079 | 33 |
| Golden Shiner | 0.017 | 2141 | 0.077 | 25 |

Information on the lethality of the golden shiner's combined temperature preference/chlorine avoidance threshold is contradictory. The threshold is below the $96HrLC_{50}$ of 0.102 mg/liter HOCl but the $LT_{50}$ at 0.077 mg/liter HOCl is only 25 hours. It must be remembered that the $LT_{50}$ is derived from a "best fit" line regressed through Heath's raw data and may not accurately project survival times. It is assumed that the $96hrLC_{50}$ more accurately represents the survival potential of the fish in chlorinated thermal discharges. In this case, the golden shiners still avoid HOCl concentrations lethal in 96 hours.

The avoidance thresholds for the channel catfish are all above the reported $96hrLC_{50}$ value (TABLE 5). Apparently the channel catfish do not avoid all lethal chlorine solutions, indicating that there is a potential for mass mortalities. On the other hand, kills of these fish have not been observed in the discharge at Glen Lyn. An explanation for these conflicting observations can be found in TABLE 6. These data show that channel catfish could survive for approximately a day and a half if exposed intermittently to chlorine doses slightly less than HOCl avoidance threshold. Most power plants achieve a maximum dose of 0.200 mg/liter TRC at the discharge canal relatively quickly and at this concentration, HOCl values generally exceed the reported avoidance thresholds. Therefore, the fish are only exposed to adverse conditions briefly before they escape the chlorinated plume.

The data indicate that the orientation of these species in relation to power plant discharges depends in part on the plant's chlorination schedule. Chlorine is generally applied intermittently to cooling waters approximately every eight hours and between chlorinations, the fish will probably be attracted to the warmer water of the thermal discharge. When the chlorine slug reaches the fish, they should be able to detect it at sublethal concentrations and retreat to more favorable locations in the environment. Therefore, these three species will not be adversely affected by power plant discharges because they will only be exposed to high chlorine concentrations for a very short time before they avoid the discharge area.

## Interim Criteria

Practically all the criteria for industrial discharges are based upon studies which considered each environmental factor independently. Thermal criteria are often based on critical thermal maxima (Otto *et al.*, 1976) and detrimental $\Delta T$ values (Schübel, 1974). Most chlorine criteria are based on studies which do not consider duration of the exposure period or the synergistic effect of temperature (Pyle, 1960; McLean, 1973; Mattice and Zittel, 1976). Much more research is needed to examine the effect of each residual chlorine constituent (TRC, CRC, FRC, and HOCl) on fish at various temperatures.

Brungs (1973) suggested that the protective criteria for chlorinated discharges be limited to approximately 0.200 mg/liter TRC for a 2-hour period each day. These criteria have been found to be adequate in certain situations (Dickson _et al._, 1974). The present study shows that such criteria provide adequate protection for those warmwater fish that demonstrate active chlorine avoidance to sub-lethal doses. The golden shiner and carp would be protected by these criteria, since they demonstrated the ability to avoid all lethal chlorine/temperature solutions.

Most of the time, the channel catfish would be protected from the chlorinated discharges, but at an acclimation temperature of 24 C the fish failed to avoid certain lethal concentrations. Catfish may be attracted to discharges abiding by the interim criteria in which the chlorine are above $LC_{50}$ values but below avoidance thresholds, indicating a potential for mortality. This situation may be averted if the criteria are amended to include HOCl criteria in addition to current TRC criteria. For all the species examined, HOCl avoidance thresholds were consistent, indicating that they may provide the information necessary for adequate standards. Presently, interim criteria appear to be inadequate and should be revised after more research on the effects of temperature synergisms and HOCl toxicity are obtained.

# VI. SUMMARY AND CONCLUSIONS

1. Many different apparatuses have been used in the past to quantify avoidance behavior of fish. For this study, a steep toxic gradient was created in the experimental troughs, because it is compact and easy to monitor. In addition, the fish could successfully orient by either kineses or taxes movements in steep gradients.

2. Various parameters were examined as possible indices of avoidance behavior. Total elapsed time within the treated vs untreated water was the simplest and most sensitive index. Time was inversely correlated with chlorine concentrations at most treatments. The mean time of each entry into treated water also varied inversely with chlorine concentrations, but differences between treatments were not significant. The number of entries into treated vs untreated waters rose and fell between chlorine treatments and was not recommended as a behavioral index.

3. Exposing fish to single chlorine doses was laborious and provided no additional information than that obtained from multiple exposures. Previous exposure to low chlorine concentrations did not significantly influence subsequent behavior. Fish were exposed to successively doubling TRC doses in all avoidance experiments.

4. The data were originally tested for differences in the residence time between target TRC concentrations and mean CRC, FRC, and HOCl concentrations were calculated. Later it was found that significant variation was introduced into the experimental data because the New River water quality fluctuated daily. The data were reanalyzed nonparametrically by each chlorine residual (CRC, FRC, and HOCl) to offset much of this variation.

5. The three species did not respond well in chlorine avoidance trials when acclimated and tested at 6 C. Movements were erratic or slow and fish frequently had to be replaced.

6. Avoidance threshold for TRC, CRC, and FRC varied with the fish's acclimation temperatures of 12, 18, 24, and 30 C. The highest thresholds generally occurred at 24 C and the lowest at 12, 18 or 30 C.

7. HOCl avoidance thresholds were very consistent between acclimation temperatures. The thresholds for carp and golden shiner varied from 0.014-0.017 and 0.015-0.017 mg/liter, respectively. The thresholds for channel catfish were slightly higher, 0.067-0.077 mg/liter.

8. The response of fish acclimated at 12 C to chlorinated waters heated to their preferred temperature was species

specific. The TRC, CRC, FRC, and HOCl avoidance thresholds for golden shiner were 0.192, 0.085, 0.101, and 0.065 mg/liter, respectively. While carp preferred every treatment, channel catfish avoided every treatment.

9. At the 24-C acclimation temperature, the TRC, CRC, and FRC avoidance thresholds for heated-chlorinated waters varied with each species. However, the HOCl threshold was similar between species -- 0.074, 0.079, and 0.077 mg/liter for the carp, channel catfish and golden shiner, respectively.

10. Chlorine avoidance behavior appeared to be controlled primarily by the HOCl content of the water. Both carp and golden shiner avoided sublethal levels of chlorine, as determined by Heath (in press). On the other hand, the channel catfish avoidance thresholds at 24 C exceeded the reported $LC_{50}$ of 0.034 mg/liter HOCl.

11. Water quality criteria based solely upon TRC concentrations may not adequately protect all warmwater fish that encounter chlorinated discharges. Interim criteria should be revised to include limits for HOCl residuals, but before adequate standards are enacted more research concerning the interaction of HOCl and temperature should be conducted on additional fish species.

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# VIII. APPENDIX

TABLE A-1: Response of carp at 6 C to steep chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | |
|---|---|---|---|---|---|---|
| | Control | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 |
| Residence Time (%) | 63.7± 7.0 | 47.8± 6.9 | 51.9± 5.9 | 47.6± 4.3 | 33.2± 9.0 | 45.3± 6.1 |
| TRC (mg/l) | 0 | .024±.001 | .056±.002 | .113±.005 | .233±.003 | .404±.012 |
| CRC (mg/l) | 0 | .024±.001 | .042±.002 | .045±.004 | .051±.003 | .061±.015 |
| FRC (mg/l) | 0 | 0 | .015±.002 | .068±.006 | .182±.005 | .343±.014 |
| HOCl (mg/l) | 0 | 0 | .010±.001 | .040±.003 | .091±.005 | .145±.008 |

TABLE A-2: Response of carp at 12 C to steep chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | |
|---|---|---|---|---|---|---|
| | Control | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 |
| Residence Time (%) | 52.0± 4.3 | 45.4± 3.8 | 33.7± 2.8 | 19.1± 3.3 | 15.0± 1.8 | 14.3± 3.1 |
| TRC (mg/l) | 0 | .023±.001 | .052±.001 | .104±.001 | .200±.004 | .403±.002 |
| CRC (mg/l) | 0 | .023±.001 | .045±.002 | .056±.003 | .072±.001 | .090±.007 |
| FRC (mg/l) | 0 | 0 | .007±.002 | .049±.002 | .128±.004 | .313±.008 |
| HOCl (mg/l) | 0 | 0 | .003±.000 | .019±.001 | .042±.005 | .090±.009 |

TABLE A-3: Response of carp at 18 C to steep chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | |
|---|---|---|---|---|---|---|
| | Control | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 |
| Residence Time (%) | 48.5± 3.5 | 44.3± 6.0 | 45.3± 5.8 | 29.5± 7.8 | 16.8± 4.7 | 21.0± 7.7 |
| TRC (mg/l) | 0 | .027±.001 | .051±.002 | .109±.002 | .211±.002 | .405±.005 |
| CRC (mg/l) | 0 | .027±.001 | .044±.001 | .059±.004 | .065±.003 | .097±.008 |
| FRC (mg/l) | 0 | 0 | .007±.002 | .050±.005 | .146±.004 | .308±.009 |
| HOCl (mg/l) | 0 | 0 | .003±.001 | .019±.001 | .044±.003 | .068±.003 |

TABLE A-4: Response of carp at 24 C to steep chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose | | | | |
|---|---|---|---|---|---|---|
| | Control | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 |
| Residence Time (%) | 50.3± 3.3 | 54.5± 6.4 | 52.6± 2.7 | 41.9± 7.0 | 16.7± 3.3 | 8.0± 1.5 |
| TRC (mg/l) | 0 | .024±.001 | .052±.002 | .108±.003 | .212±.004 | .404±.004 |
| CRC (mg/l) | 0 | .024±.001 | .046±.003 | .076±.005 | .074±.007 | .139±.008 |
| FRC (mg/l) | 0 | 0 | .006±.000 | .032±.005 | .138±.008 | .265±.008 |
| HOCl (mg/l) | 0 | 0 | .002±.000 | .010±.001 | .035±.003 | .062±.008 |

TABLE A-5: Response of carp at 30 C to steep chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | |
|---|---|---|---|---|---|---|
| | Control | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 |
| Residence Time (%) | 55.8± 2.0 | 46.3± 4.2 | 40.4± 6.3 | 21.0± 2.8 | 7.5± 2.0 | 3.6± 1.2 |
| TRC (mg/l) | 0 | .023±.001 | .054±.001 | .106±.004 | .197±.003 | .409±.003 |
| CRC (mg/l) | 0 | .023±.001 | .050±.002 | .082±.002 | .088±.008 | .131±.019 |
| FRC (mg/l) | 0 | 0 | .004±.001 | .024±.005 | .110±.010 | .278±.017 |
| HOCl (mg/l) | 0 | 0 | .002±.000 | .010±.002 | .041±.004 | .087±.003 |

TABLE A-6: Response of channel catfish at 6 C to steep chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | |
|---|---|---|---|---|---|---|
| | Control | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 |
| Residence Time (%) | 55.0± 2.8 | 55.7± 4.2 | 59.3± 3.0 | 54.7± 3.0 | 48.3± 3.3 | 52.0± 9.0 |
| TRC (mg/l) | 0 | .024±.001 | .060±.001 | .117±.005 | .222±.004 | .423±.007 |
| CRC (mg/l) | 0 | .024±.001 | .047±.002 | .058±.001 | .098±.012 | .091±.010 |
| FRC (mg/l) | 0 | 0 | .013±.002 | .060±.005 | .125±.013 | .332±.014 |
| HOCl (mg/l) | 0 | 0 | .009±.002 | .032±.002 | .050±.003 | .111±.006 |

TABLE A-7: Response of channel catfish at 12 C to steep chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | |
|---|---|---|---|---|---|---|
| | Control | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 |
| Residence Time (%) | 53.0± 2.6 | 46.3± 4.0 | 48.2± 3.6 | 40.9± 9.1 | 37.6± 6.6 | 32.9±11.1 |
| TRC (mg/l) | 0 | .025±.002 | .052±.001 | .111±.001 | .207±.002 | .400±.002 |
| CRC (mg/l) | 0 | .025±.002 | .049±.002 | .070±.005 | .089±.005 | .129±.002 |
| FRC (mg/l) | 0 | 0 | .003±.000 | .040±.005 | .118±.005 | .271±.002 |
| HOCl (mg/l) | 0 | 0 | .002±.000 | .024±.003 | .060±.002 | .114±.009 |

TABLE A-8: Response of channel catfish at 18 C to steep chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose | | | | |
|---|---|---|---|---|---|---|
| | Control | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 |
| Residence Time (%) | 53.1± 2.8 | 47.4± 5.1 | 39.1± 6.6 | 41.9± 5.1 | 30.0± 7.4 | 16.1± 4.3 |
| TRC (mg/l) | 0 | .025±.001 | .051±.001 | .099±.002 | .209±.002 | .411±.003 |
| CRC (mg/l) | 0 | .025±.001 | .048±.001 | .072±.002 | .099±.008 | .135±.013 |
| FRC (mg/l) | 0 | 0 | .003±.000 | .027±.002 | .111±.006 | .276±.012 |
| HOCl (mg/l) | 0 | 0 | .001±.000 | .011±.000 | .038±.003 | .078±.007 |

TABLE A-9: Response of channel catfish at 24 C to steep chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | |
|---|---|---|---|---|---|---|
| | Control | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 |
| Residence Time (%) | 49.1±.2.3 | 51.2± 5.2 | 55.1± 7.5 | 42.3± 9.1 | 30.5± 8.3 | 17.2± 3.8 |
| TRC (mg/l) | 0 | .024±.001 | .050±.001 | .102±.002 | .203±.002 | .403±.001 |
| CRC (mg/l) | 0 | .024±.001 | .047±.001 | .073±.002 | .107±.008 | .184±.044 |
| FRC (mg/l) | 0 | 0 | .003±.000 | .030±.00 | .096±.009 | .219±.016 |
| HOCl (mg/l) | 0 | 0 | .001±.000 | .013±.000 | .033±.002 | .066±.002 |

TABLE A-10: Response of channel catfish at 30 C to steep chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | |
|---|---|---|---|---|---|---|
| | Control | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 |
| Residence Time (%) | 55.8± 1.9 | 53.5± 4.3 | 66.5± 4.0 | 53.2± 2.9 | 20.0± 2.2 | 10.3± 4.1 |
| TRC (mg/l) | 0 | .022±.001 | .051±.001 | .105±.002 | .205±.003 | .400±.004 |
| CRC (mg/l) | 0 | .022±.001 | .050±.002 | .072±.003 | .097±.003 | .142±.011 |
| FRC (mg/l) | 0 | 0 | .002±.001 | .033±.004 | .108±.006 | .258±.011 |
| HOCl (mg/l) | 0 | 0 | .001±.000 | .013±.001 | .037±.001 | .077±.001 |

TABLE A-11: Response of golden shiners at 6 C to steep chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 | 0.800 |
| Residence Time (%) | 46.7± 3.0 | 43.9± 3.5 | 45.0± 2.5 | 39.4± 2.9 | 32.4±11.4 | 18.5± 3.9 | 15.8± 7.7 |
| TRC (mg/l) | 0 | .024±.001 | .048±.002 | .107±.003 | .211±.004 | .430±.006 | .821±.009 |
| CRC (mg/l) | 0 | .023±.001 | .036±.001 | .050±.005 | .073±.011 | .078±.015 | .109±.024 |
| FRC (mg/l) | 0 | 0 | .011±.002 | .057±.007 | .138±.012 | .351±.011 | .712±.028 |
| HOCl (mg/l) | 0 | 0 | .007±.001 | .034±.006 | .069±.009 | .163±.011 | .298±.040 |

TABLE A-12: Response of golden shiners at 12 C to steep chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 | 0.800 |
| Residence Time (%) | 51.0± 1.4 | 53.3± 2.1 | 48.3± 4.0 | 36.1± 6.1 | 24.0± 6.2 | 18.1± 4.2 | 7.9± 2.2 |
| TRC (mg/l) | 0 | .025±.001 | .052±.002 | .108±.004 | .209±.003 | .402±.005 | .805±.003 |
| CRC (mg/l) | 0 | .022±.001 | .046±.002 | .069±.004 | .086±.007 | .136±.023 | .272±.053 |
| FRC (mg/l) | 0 | .003±.000 | .005±.000 | .039±.002 | .123±.010 | .265±.026 | .532±.054 |
| HOCl (mg/l) | 0 | .002±.000 | .003±.000 | .018±.001 | .044±.004 | .069±.007 | .126±.014 |

TABLE A-13: Response of golden shiners at 18 C to steep chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 | 0.800 |
| Residence Time (%) | 52.7± 2.2 | 49.0± 2.9 | 55.3± 6.3 | 44.4± 8.9 | 28.9± 9.2 | 17.3± 5.7 | 6.6± 2.2 |
| TRC (mg/l) | 0 | .025±.001 | .050±.002 | .101±.002 | .199±.005 | .396±.006 | .790±.004 |
| CRC (mg/l) | 0 | .023±.001 | .044±.002 | .066±.004 | .112±.004 | .135±.014 | .229±.026 |
| FRC (mg/l) | 0 | .001±.000 | .005±.002 | .035±.005 | .086±.007 | .261±.017 | .561±.024 |
| HOCl (mg/l) | 0 | .001±.000 | .003±.001 | .014±.001 | .027±.003 | .073±.007 | .132±.015 |

TABLE A-14: Response of golden shiners at 24 C to steep chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 | 0.800 |
| Residence Time (%) | 54.1± 2.6 | 66.1± 5.1 | 72.7± 9.0 | 54.6±10.3 | 32.6± 6.5 | 16.6±11.9 | 11.0± 7.2 |
| TRC (mg/l) | 0 | .025±.001 | .050±.002 | .100±.003 | .205±.005 | .395±.022 | .800±.003 |
| CRC (mg/l) | 0 | .026±.001 | .051±.002 | .085±.006 | .158±.011 | .255±.022 | .497±.039 |
| FRC (mg/l) | 0 | 0 | 0 | .015±.005 | .047±.007 | .139±.021 | .303±.039 |
| HOCl (mg/l) | 0 | 0 | 0 | .006±.001 | .017±.003 | .045±.008 | .093±.015 |

TABLE A-15: Response of golden shiners at 30 C to steep chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 | 0.800 |
| Residence Time (%) | 52.8± 2.9 | 49.3± 7.9 | 50.6± 8.1 | 38.7± 3.2 | 24.5± 2.6 | 8.7± 4.7 | 5.5± 3.0 |
| TRC (mg/l) | 0 | .023±.001 | .052±.002 | .103±.003 | .203±.004 | .404±.003 | .799±.002 |
| CRC (mg/l) | 0 | .023±.001 | .049±.002 | .087±.002 | .114±.010 | .146±.018 | .277±.039 |
| FRC (mg/l) | 0 | 0 | .003±.001 | .016±.002 | .088±.008 | .258±.021 | .522±.041 |
| HOCl (mg/l) | 0 | 0 | .001±.000 | .006±.000 | .033±.004 | .081±.011 | .136±.018 |

TABLE A-16: Response of carp at 12 C to steep temperature/chlorine gradients (Mean ± S.E.).

| | Control | Target Chlorine Dose (mg/l) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0.000 | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 |
| Residence Time (%) | 53.7± 1.6 | 74.4± 4.8 | 79.1± 6.4 | 59.5± 8.3 | 61.2± 7.4 | 56.7± 9.0 | 74.6± 7.4 |
| TRC (mg/l) | 0 | 0 | .027±.001 | .057±.001 | .104±.002 | .201±.002 | .404±.005 |
| CRC (mg/l) | 0 | 0 | .027±.001 | .053±.000 | .087±.003 | .086±.003 | .110±.008 |
| FRC (mg/l) | 0 | 0 | 0 | .003±.000 | .016±.003 | .115±.004 | .294±.011 |
| HOCl (mg/l) | 0 | 0 | 0 | .002±.000 | .006±.001 | .030±.001 | .055±.002 |

TABLE A-17: Response of carp at 24 C to steep temperature/chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 0.000 | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 |
| Residence Time (%) | 45.8± 3.1 | 63.6± 5.4 | 64.4± 5.6 | 65.7± 6.2 | 53.8± 5.6 | 25.4± 6.1 | 10.0± 3.0 |
| TRC (mg/l) | 0 | 0 | .025±.000 | .056±.001 | .106±.002 | .205±.004 | .408±.005 |
| CRC (mg/l) | 0 | 0 | .024±.000 | .054±.001 | .093±.003 | .143±.009 | .176±.029 |
| FRC (mg/l) | 0 | 0 | 0 | .001±.000 | .012±.003 | .062±.009 | .232±.034 |
| HOCl (mg/l) | 0 | 0 | 0 | 0 | .004±.000 | .015±.003 | .047±.008 |

TABLE A-18: Response of channel catfish at 12 C to steep temperature/chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 0.000 | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 |
| Residence Time (%) | 48.5± 1.5 | 68.5± 4.4 | 55.9± 8.6 | 54.0± 9.5 | 20.4± 8.6 | 23.6± 8.1 | 15.4± 9.5 |
| TRC (mg/l) | 0 | 0 | .024±.001 | .055±.000 | .107±.012 | .204±.003 | .407±.003 |
| CRC (mg/l) | 0 | 0 | .023±.000 | .048±.002 | .076_.002 | .111±.006 | .145±.007 |
| FRC (mg/l) | 0 | 0 | .001±.000 | .007±.002 | .031±.002 | .093±.009 | .262±.009 |
| HOCl (mg/l) | 0 | 0 | 0 | .004±.001 | .014±.001 | .035±.003 | .081±.003 |

TABLE A-19: Response of channel catfish at 24 C to steep temperature/chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 0.000 | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 |
| Residence Time (%) | 50.1± 1.6 | 37.8± 7.9 | 28.3± 9.6 | 22.1± 6.7 | 16.5± 5.3 | 9.2±5.2 | 4.6± 3.7 |
| TRC (mg/l) | 0 | 0 | .025±.000 | .048±.001 | .097±.002 | .205±.002 | .401±.002 |
| CRC (mg/l) | 0 | 0 | .024±.000 | .043±.001 | .073±.002 | .099±.003 | .168±.010 |
| FRC (mg/l) | 0 | 0 | 0 | .004±.000 | .023±.003 | .105±.005 | .233±.012 |
| HOCl (mg/l) | 0 | 0 | 0 | .002±.000 | .010±.001 | .037±.001 | .066±.002 |

TABLE A-20: Response of golden shiners at 12 C to steep temperature/chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 0.000 | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 |
| Residence Time (%) | 55.7± 2.9 | 81.2± 4.6 | 72.7± 4.2 | 76.0± 3.4 | 37.5± 7.0 | 22.1± 4.5 | 24.7± 8.7 |
| TRC (mg/l) | 0 | 0 | .024±.000 | .047±.002 | .099±.004 | .192±.003 | .392±.007 |
| CRC (mg/l) | 0 | 0 | .022±.000 | .042±.001 | .062±.001 | .082±.005 | .127±.006 |
| FRC (mg/l) | 0 | 0 | .001±.000 | .005±.001 | .036±.004 | .110±.006 | .264±.011 |
| HOCl (mg/l) | 0 | 0 | .000±.000 | .003±.000 | .022±.003 | .060±.0 4 | .125±.007 |

TABLE A-21: Response of golden shiners at 24 C to steep temperature/chlorine gradients (Mean ± S.E.).

| | | Target Chlorine Dose (mg/l) | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 0.000 | 0.025 | 0.050 | 0.100 | 0.200 | 0.400 |
| Residence Time (%) | 50.7± 3.4 | 62.2± 3.8 | 63.2± 5.4 | 53.1± 2.8 | 43.6± 5.1 | 7.1± 2.4 | 6.2± 2.6 |
| TRC (mg/l) | 0 | 0 | .025±.001 | .048±.001 | .107±.003 | .217±.012 | .400±.003 |
| CRC (mg/l) | 0 | 0 | .025±.001 | .043±.001 | .075±.002 | .107±.005 | .175±.007 |
| FRC (mg/l) | 0 | 0 | 0 | .005±.000 | .031±.004 | .110±.009 | .225±.007 |
| HOCl (mg/l) | 0 | 0 | 0 | .003±.000 | .015±.002 | .049±.005 | .091±.005 |

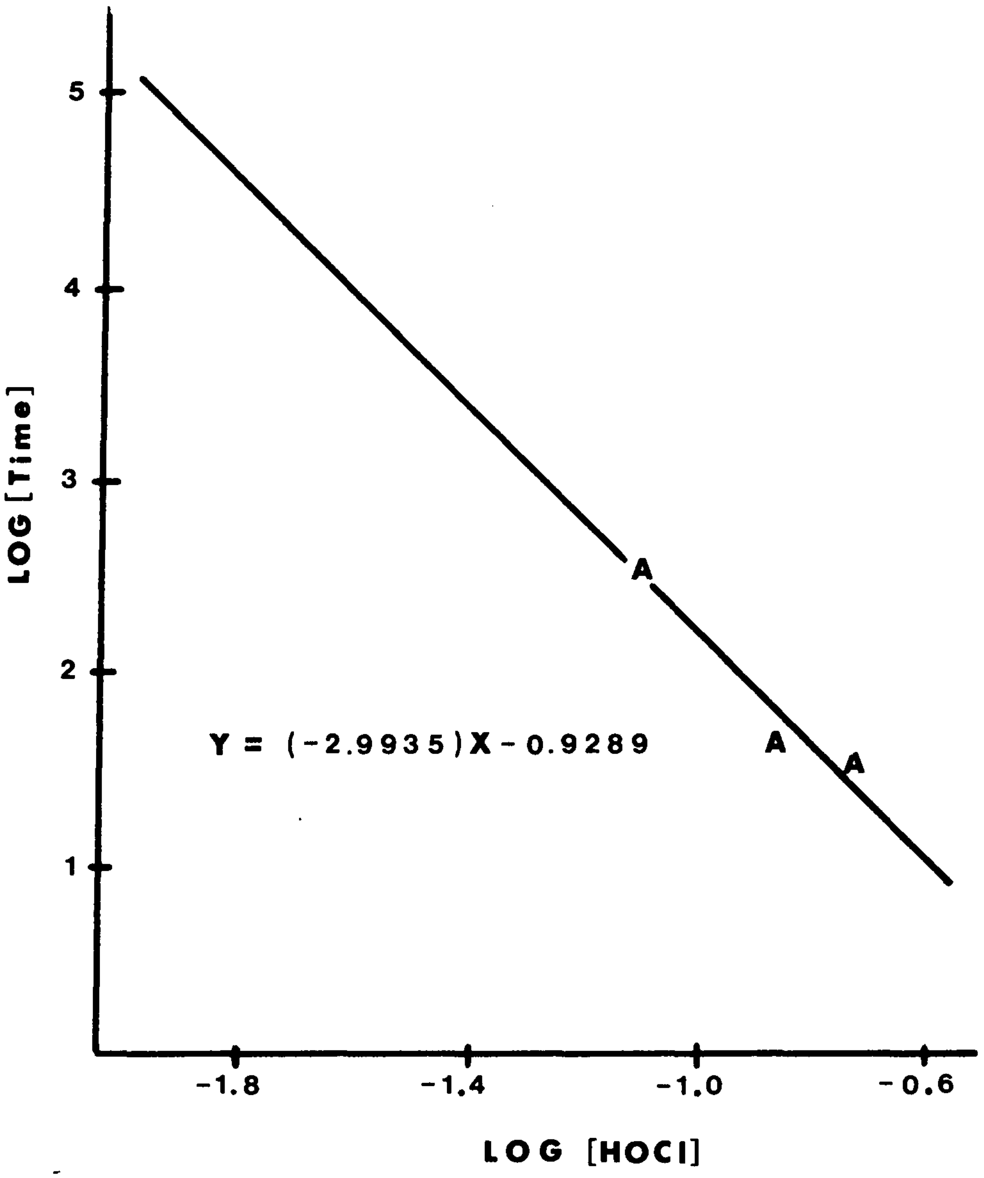

FIGURE A-1: "Best Fit" regression line used to calculate median survival times of golden shiners at 24 C to chlorine avoidance thresholds (calculated from Heath, in press).

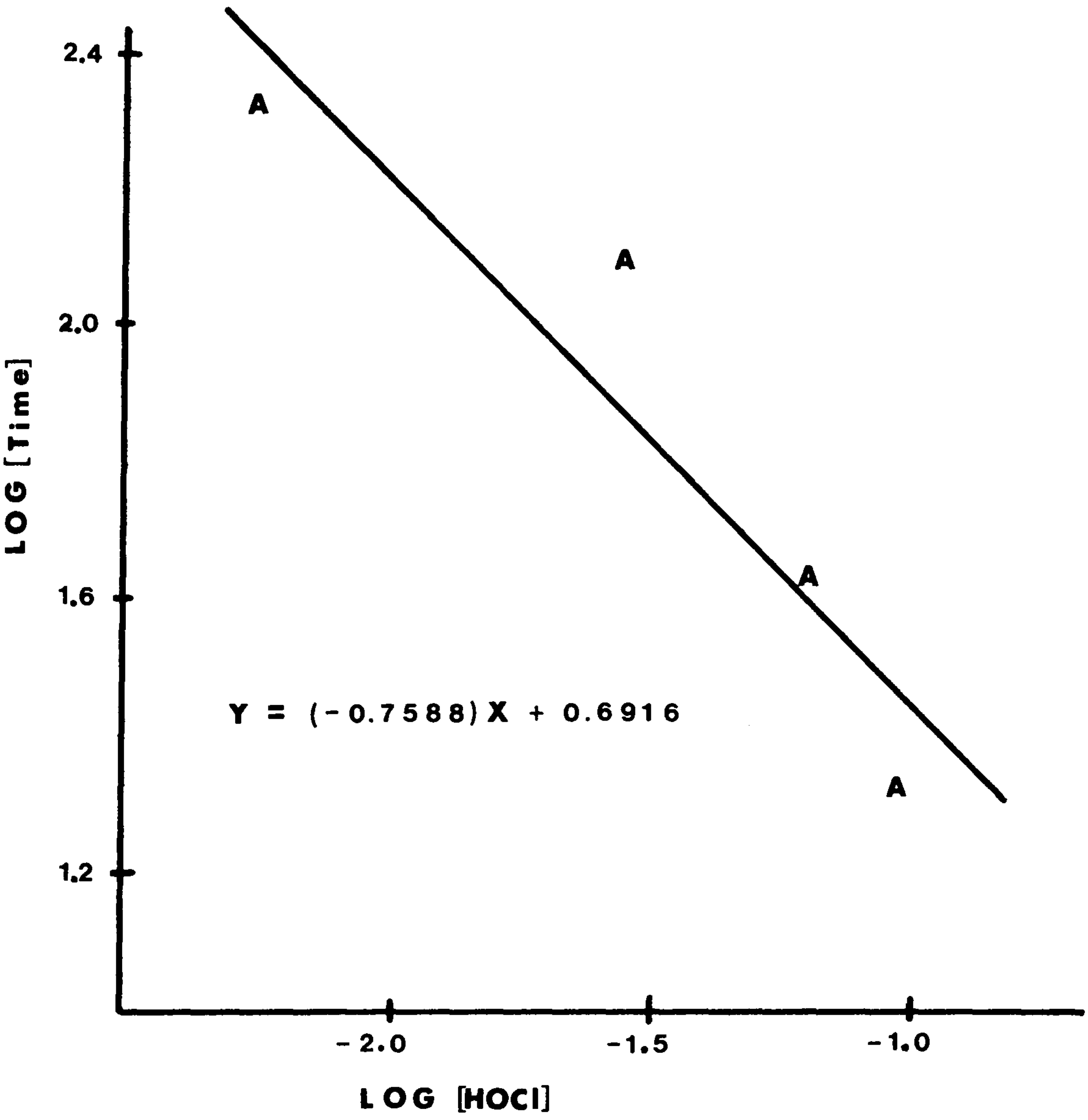

FIGURE A-2: "Best Fit" regression line used to calculate median survival times of channel catfish at 24 C to chlorine avoidance thresholds (calculated from Heath, in press).

# BEHAVIORAL AVOIDANCE BY FISH OF RESIDUAL CHLORINE IN POWER PLANT DISCHARGES

BY

Stephan Richard Larrick

## (ABSTRACT)

The behavior of fishes in and around power plant discharges has not been adequately explored despite the recent proliferation of electric generating stations. This study investigated the response of fish to heated and/or chlorinated waters in a field laboratory. Carp (Cyprinus carpio), channel catfish (Ictalurus punctatus) and golden shiners (Notemigonus crysoleucas) were tested at 6, 12, 18, 24 and 30-C acclimation temperatures in a steep concentration gradient. Residence time in the treated water was determined to be the most simple and sensitive index of avoidance behavior. TRC, CRC, and FRC avoidance thresholds varied between species and acclimation temperatures. HOCl avoidance thresholds were consistent between acclimation temperatures, suggesting that HOCl is the primary constituent of the TRC controlling avoidance behavior.

Trials were conducted at 12 and 24 C in which the treated water was heated to a preferred temperature, derived in a shallow horizontal temperature gradient. At low chlorine doses fish significantly preferred the warmer water, whereas at high chlorine doses, the fish were repelled by the treated water.

The TRC, CRC, and FRC avoidance thresholds varied between fish species, but the HOCl avoidance thresholds were similar for all species at 24 C. The avoidance thresholds for the carp and golden shiners were well below 96hrLC$_{50}$ values, signifying that they can avoid dangerous solutions before they are adversely affected. The channel catfish avoidance thresholds are slightly greater than reported 96hrLC$_{50}$ values, indicating a potential for mortalities in power plant discharges. This mortality has not been observed in the field and alternate explanations are discussed.

Present interim criteria for chlorinated discharges may not adequately protect all warmwater fish. This study indicates that HOCl may greatly influence fish behavior and suggests that HOCl criteria be adopted for use in conjunction with current total residual chlorine criteria.