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Treflan and the Oxygen Consumption of Green Sunfish (Lepomis cyanellus)

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Treflan and the oxygen consumption of

green sunfish (Lepomis cyanellus)
(TITLE)

BY

Barbara Jo Warner
B. S. in Biological Science
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THESIS

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I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
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TREFLAN AND THE OXYGEN CONSUMPTION OF GREEN SUNFISH (*LEPOMIS CYANELLUS*)

Presented by

BARBARA JO WARNER

a candidate for the degree of Master of Science

and hereby certify that in their opinion it is acceptable.

ABSTRACT

Oxygen consumption rates of 51 green sunfish (Lepomis cyanellus, Rafinesque) were monitored over five hours exposure to Treflan E.C. concentrations of 0, 0.32, 0.56, 1.0, and 2.0 ppm. There was a great variation in the responses of the fish at each concentration; and Student's t-tests revealed no significant differences. However, graphs of the mean hourly oxygen consumption rates showed certain trends. During the fifth hour of exposure, there appears to be an acclimation to the Treflan at concentrations of 0.32 and 0.56 ppm; but no such acclimation at the higher concentrations of 1.0 and 2.0 ppm. Hourly oxygen consumption rates at 2.0 ppm Treflan fluctuated less than at the other concentrations. Also, Treflan concentrations of 0.56, 1.0, and 2.0 ppm altered the consumption rates of the larger fish more than those of the smaller fish.

Further tests with longer periods of exposure to Treflan are recommended to determine further reactions to the higher Treflan concentrations.

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ACKNOWLEDGEMENTS

I would like to thank Dr. L. S. Whitley for the suggestion and initial guidance in this study; and Dr. L. Durham for continued guidance and evaluation.

I am also grateful to Drs. Brigham and Childers of the Illinois Natural History Survey for supplying the fish; and to Dr. R. C. Hiltibran for the use of his reference material. Thanks are due Drs. M. Chapman, R. D. Darding, and W. J. Keppler for critical review of the manuscript. Thanks are also due Dr. A. J. DiPietro and Kurt Clement for their help with statistical analysis.

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LITERATURE REVIEW

Trifluralin

Trifluralin is a popular soil-incorporated herbicide used in the preemergence control of a number of annual grasses and broadleaf weeds. This herbicide is marketed by the Elanco Products Company under the registered trade name of Treflan. It is available in two forms. Treflan E.C. is a 4 lb./gallon emulsifiable concentrate (E.C.) of 44.5% active ingredients. It is mixed with water and applied at rates ranging from 1 to $2\frac{1}{2}$ pints per acre. Also available is a 5% granular formulation.

Pure trifluralin (a,a,a-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) (Fig. 1) is soluble in acetone, xylene, and aromatic napthas. Its solubility in water is less than 1 ppm at 27°C. The vapor pressure of trifluralin is 1.99×10^{-4} mm Hg at 29.5°C, and the boiling point is 96-97°C at 0.18 mm Hg (Elanco 1968 and 1973).

Studies to determine mechanisms of action have shown that trifluralin affects the root development of seedlings. Treated roots assume a club-like appearance because they fail to elongate normally, while continuing radial expansion near the root tip (Bayer et al. 1967, Hacskaylo and Amato 1968, Lignowski and Scott 1971). The cells at the extreme tip of treated roots are usually small and dense, whereas behind this area, the cells become abnormally large and thin-walled (Hacskaylo and Amato 1968).

Investigations of chromatin activity have shown that trifluralin has a definite inhabitory effect on mitosis (Lignowski and Scott

1972, Penner and Early 1972, Talbert 1965). It appears to act in much the same manner as colchicine, by disrupting the spindle apparatus and thus blocking mitosis at metaphase (Lignowski and Scott 1972). Trifluralin also markedly decreases the synthesis of RNA in corn seedlings. There is evidence that it binds to the chromatin, thus reducing the availability of a template for transcription (Penner and Early 1972). In addition, Negi et al. (1968) found that trifluralin inhibits the oxygen uptake and oxidative phosphorylation in isolated mitochondria of corn, sorghum, and soybean. This study indicated that phosphate uptake can decrease 50% or more in the presence of 10^{-4}M trifluralin.

Trifluralin has been shown to be subject to photodecomposition and shows a progressive decrease in phytotoxicity with increasing time and intensity of exposure to ultraviolet rays (Wright and Warren 1965). However, the losses from photodecomposition are significantly reduced when it is applied to the soil. There is greater volatility of trifluralin with increasing temperature and increasing soil moisture (Parochetti and Hein 1973).

The persistence and phytotoxicity of trifluralin are determined by several factors, such as time of application, depth and method of incorporation, and type of soil. Trifluralin exerts its maximum phytotoxic action only when it is applied shortly before or after sowing (Casarini 1968, Smith and Wiese 1973). Soil bioassays have shown that the greatest retention to trifluralin is derived by disking treated plots before bedding. Application and shallow incorporation after planting result in reduced retention with volatilization, photodecomposition, and desorption probably causing the dissipation of the trifluralin (Oliver

and Frans 1968, Savage and Barrentine 1969, Menges and Tamez 1974).

According to Parka and Worth (1965), degredation studies using C^{14} labeled trifluralin have shown greater than 90% trifluralin remaining after three months. They suggest that this shows a negligible accumulation of degredation products which could be harmful to fish or other wildlife. However, other studies have shown that, at normal incorporation depths, only residual levels of trifluralin persist after three to four months (Savage 1973, Axe et al. 1969). Thus there is no accumulation with repeated annual applications (Parka and Tepe 1969, Savage 1973). Probst et al. (1967) found that trifluralin decreased to a 10% to 15% concentration within 6 to 12 months. This field study revealed different degredative pathways in the soil. Under aerobic conditions, the main pathway is dealkylation followed by progressive reduction (Fig. 2). In an anaerobic situation (under conditions of excessive rainfall and poor drainage), the degredative pathway would be one of reduction followed by dealkylation (Fig. 3).

Trifluralin Toxicity to Fish

Static water tests have shown that trifluralin is very toxic to certain species of fish. Latvaitis (1972) observed the reactions of 20 species to trifluralin concentrations of 0.75, 1.275, and 1.8 ppm. A cichlid, Cichlasoma centrarchus, was the most resistant of these species, with LC_0s at 0.75 and 1.8 ppm. The only centrarchid species tested, Lepomis macrochirus (Rafinesque), proved to be the most sensitive, with LC_{100}s at all three concentrations. Latvaitis observed a pronounced difference between experimental and control fish within two hours after addition of Treflan E.C. He suggests that the loss of equilibrium,

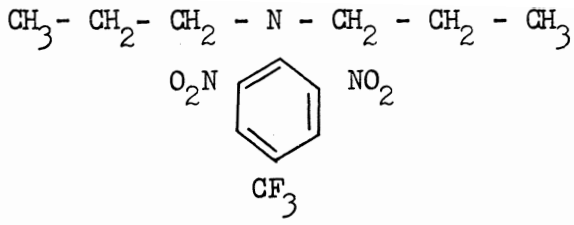

Fig. 1. a,a,a-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine.

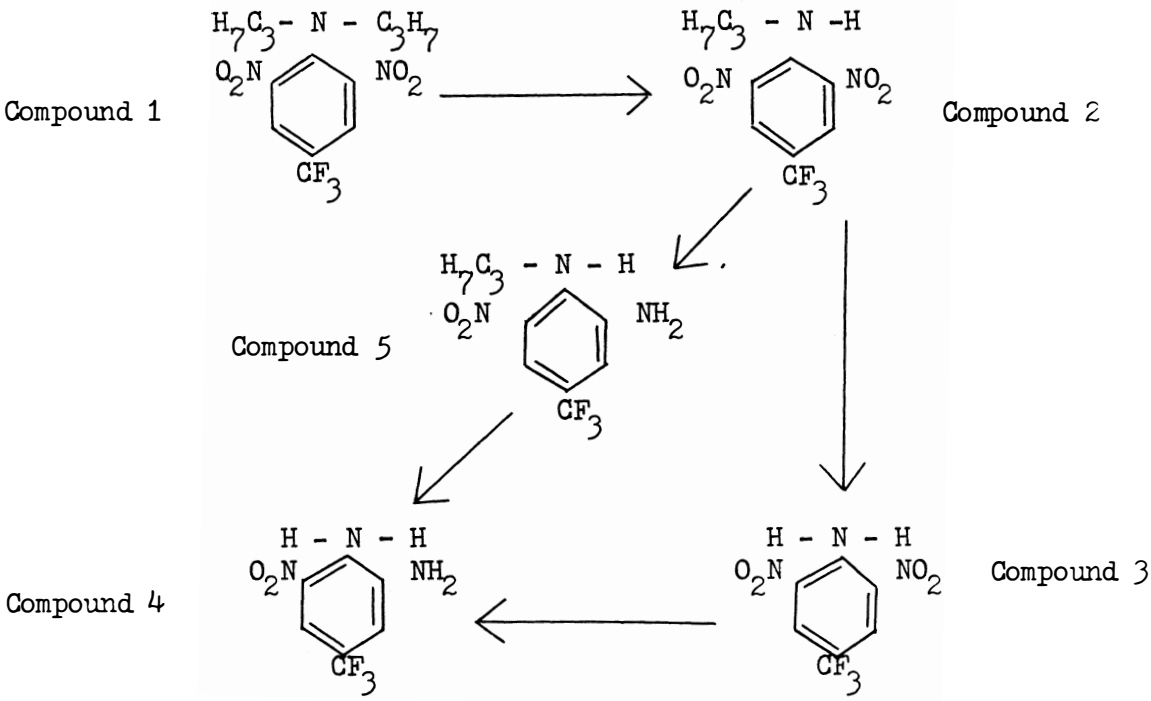

Fig. 2. Postulated pathway of aerobic trifluralin degredation (Probst et al. 1967).

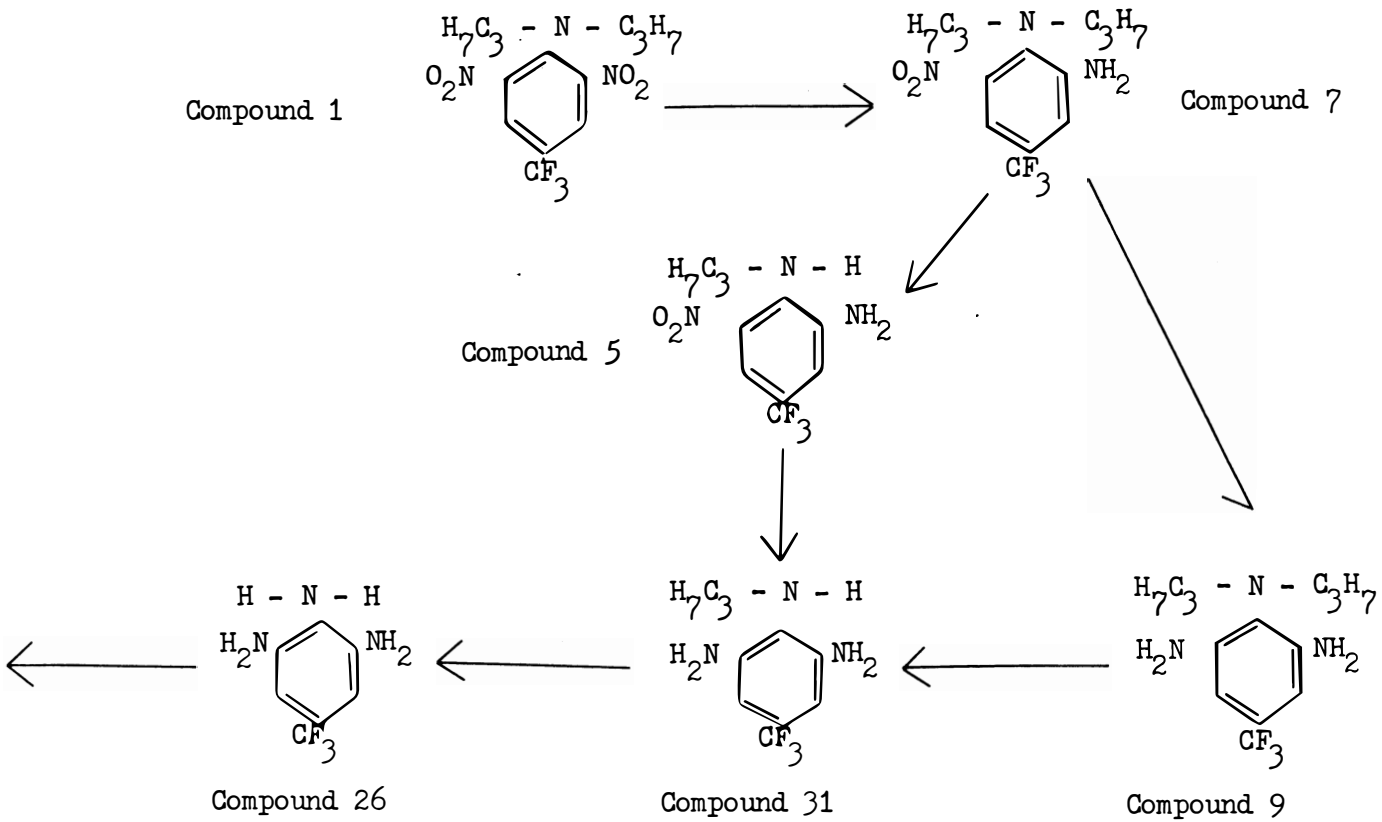

Fig. 3. Postulated pathway of anaerobic trifluralin degredation (Probst et al. 1967).

excessive irritability, and convulsive movements displayed by experimental fish indicate that trifluralin affects the nervous system. He states that the excitability and hurried responses could cause increased metabolism, and therefore, respiratory increases and irregularities. Affected fish also showed scoliosis and hematoma in varying degrees; and "recovery following exposure to Treflan E.C. was almost irreversible in most families."

In similar test to determine the toxicity of trifluralin to fish, Parka and Worth (1965) found an LC_{50} of 58.2 ppb for bluegills. This concentration was within the range found by the Fish-Pesticide Research Laboratory at Denver, Colorado (23 to 120 ppb). Tests on fathead minnows gave a LC_{50} of 93.4 ppb; while goldfish had an LC_{50} of 58.5 ppb. However, when put on the soil and then added to water, 48 to 227 times more trifluralin was required to obtain an LC_{50}.

Herbicide toxicity to fish may be influenced by a number of parameters. It has been demonstrated that time and temperature affect the toxic responses of fish to trifluralin (Table 1). Parameters such as the hardness of the water and the dissolved oxygen concentration have been shown to produce a definite effect on the toxicities of various herbicides. Generally, herbicides are more toxic in soft water than in hard water (Surber and Pickering 1962, Hughes and Davis 1963); and survival time decreases as the dissolved oxygen concentration decreases (Lloyd 1961, Downing 1953).

Hughes and Davis (1962) found in testing 10 herbicides (not including trifluralin) that most were more toxic as liquid formulations than as granular formulations. Hiltibran (1967) had similar results in his tests on the

effects of herbicides on fertilized fish eggs and fry.

Table 1. Effects of time and temperature on the toxicity of trifluralin to bluegill averaging 38mm in length and 0.89g. in weight (Cope 1965).

temperature	LC_{50} micrograms/liter		
F	24 hours	48 hours	96 hours
85	10	8.4	8.4
75	120	66	47
65	360	200	135
55	530	380	210
45	1300	590	280

Oxygen Consumption

Because of the direct relation between activity and oxygen uptake in fish, three oxygen consumption levels have been defined. These are standard, routine, and active oxygen consumption (Fry, in Brown 1957). The standard level is defined as the oxygen uptake in the absence of spontaneous activity--or the "nearest attainable approximation to the basal metabolism". The routine level is the oxygen uptake when the only movements of the fish are spontaneous; and the active level is defined as the uptake when the fish is stimulated to its maximum activity (Beamish and Mookherjii 1964).

In addition to activity, many other factors may influence the oxygen consumption of fishes. These include: ambient concentrations of oxygen and carbon dioxide, temperature of the water, possible diurnal or seasonal patterns, the size of the fish, and the presence of pollutants. Oxygen and carbon dioxide concentrations may influence either the frequency or amplitude of respiratory movements. Oxygen lack, in general, tends to increase both the frequency and amplitude; while a carbon dioxide excess causes an increase in the amplitude with only a slight increase in frequency (Randall and Shelton 1963). While the standard rate of oxygen consumption does not change significantly with increasing carbon dioxide concentrations until the lower lethal limit of oxygen is reached (Black et al. 1954, Beamish 1964c, Moss and Scott 1961), the active oxygen consumption decreases logarithmically with increasing carbon dioxide concentrations (Basu 1959, Beamish 1964c). The linear relation is characteristic of a given species (Basu 1959).

Investigations have shown that both the weight of the fish and the temperature of the surrounding water have a direct effect on oxygen uptake. As expected, larger individuals consume more oxygen than smaller individuals. However, the oxygen consumption per unit weight is greater for the smaller fish. Temperature increases cause corresponding increases in oxygen consumption (Belding 1929, Penez and Prokes 1973, O'Hara 1968, Beamish and Mookherjii 1964, Moss and Scott 1961).

Higginbotham (1947) found evidence of an endogenous activity or diurnal pattern in madtom and Southern channel catfish. The fish consumed more oxygen in the late afternoon than in the morning or mid-day. However, Moss and Scott (1961) found no evidence of consistent diurnal

patterns in bluegill, largemouth bass, and channel catfish.

In a study concerning the standard rate of oxygen consumption for brook trout and brown trout, Beamish (1964b) found that the consumption varied seasonally. This was due to greater oxygen need during spawning with the higher oxygen uptake values occurring during the fall spawning period.

Starvation will result in a decrease in oxygen consumption, because of decreased need for oxygen in the assimilation of food. Beamish (1964a) found that both standard and routine oxygen consumption for Salvelinus fontinalis and Catostomus commersoni decreased with starvation for two or three days until it reached a constant level.

Pollution and Oxygen Consumption

Some toxicants (cyanides and sulfides), which inhibit respiration at the tissues, cause a progressive reduction in oxygen consumption accompanied by a corresponding decrease in the rate of opercular movement. The close agreement between oxygen consumption and opercular movement is caused by the decrease in the production of carbon dioxide. Heavy metal salts, which obstruct respiration at the gill surfaces, produce an initial increase in respiratory rate. However, as the oxygen consumption decreases, the opercular rate increases and then falls rapidly when oxygen intake reaches approximately 38% normal. In this case, the increased carbon dioxide content in the blood stimulates the respiratory center, causing respiratory movements to increase. The fish, eventually exhausted, dies due to insufficient oxygen (Jones 1946).

When DDT or kraft mill effluents are present, there is a direct relationship between respiratory irregularity and toxicant concentration. The frequency of coughing, or reversing the flow of water over

the gills, increases in direct relation to the toxicant concentration (Schaumberg et al. 1967, Walden et al. 1970). Walden et al. (1970) found that rainbow trout showed one respiratory pattern at high concentrations of effluents, and another pattern at sublethal concentrations. At toxic concentrations, there was a steady increase in the cough rate which then became erratic and terminated in death. However, at sublethal concentrations, the cough rate immediately increased, reached a maximum and then decreased slowly to merge with the base rate.

MATERIALS AND METHODS

Green sunfish (Lepomis cyanellus, Rafinesque), weighing 1.3 to 5.3 grams, were obtained from the Illinois Natural History Survey ponds in Urbana, Illinois. They were obtained in lots of two to three dozen and held in a 650 liter laboratory tank for two weeks before testing. The tank contained deionized water (Table 2) which ranged in temperature from 21 to 25C. Continuous aeration with carborundum airstones maintained the oxygen concentration at 90% or above. The fish were fed Tetramin commercial fish food daily.

The fish were starved for 48 hours prior to each experiment to allow the routine oxygen consumption to reach a constant level (Beamish 1964a). They were then weighed and placed individually in one-liter Erlenmeyer flasks. Each flask contained deionized water which had been aerated for 12 hours. The flasks were transferred to a Sherer Controlled Environmental Lab (Model Cel 257, Sherer-Gillett Co.) where the air temperature was maintained at 20 $\pm$ 1 C. The fish were allowed to acclimate to the flasks for one hour. At this time, the airstones were removed and Treflan E.C. was added to the desired concentration. An oxygen/temperature probe (Y.S.I. 5400 series) was placed in the flask, which was sealed with a rubber cork coated with petroleum jelly. Aluminum foil was also placed over the cork and top of the flask and sealed tightly. Each flask was immediately placed on a magnetic mixer set at the minimum speed. The oxygen concentration and water temperatures were recorded, using a Y.S.I. model 54 oxygen meter. Since each of the probes calibrated

differently, the initial oxygen concentration reading was assigned a value of 100% air saturation.

Readings of oxygen concentration and water temperature were recorded at 30 minute intervals for five hours, giving a total of 11 readings for each fish. Any behavior judged significant was also recorded.

Treflan E.C. concentrations used were 0, 0.32, 0.56, 1.0, and 2.0 ppm. The Treflan was stored in a dark bottle in the laboratory to prevent photodecomposition (Wright and Warren 1965). A stock solution of 200 microliters Treflan E.C. to one liter of deionized water (200 ppm) was made each day. This was then diluted to the desired concentrations (Table 3). Eleven fish were tested at 0 ppm; and 10 fish were tested at each of the other concentrations.

Table 2. Deionized water used as a medium for green sunfish exposed to various trifluralin concentrations.

content	concentration
sulfate	80 ppm
nitrate	.5 ppm
hardness - calcium	60 mg/l
- total	120 mg/l

Table 3. Dilution factors used to obtain desired trifluralin concentrations. Stock solution is 200 ppm.

Desired concentration	stock solution diluted to 100 ppm	diluted solution put in with fish
2 ppm	------	10 ml. (stock solution)
1 ppm	50 ml.	10 ml.
.56 ppm	28 ml.	10 ml.
.32 ppm	16 ml.	10 ml.

RESULTS

A plot of cumulative oxygen consumption per gram against time shows no progressive trend with increased Treflan concentrations (Fig. 4). However, when the hours of exposure are considered separately, certain relationships are indicated.

Fluctuations in oxygen consumption with increasing time of exposure appear to decrease at the higher Treflan concentrations (Fig. 5). At 2.0 ppm, the oxygen consumption was very steady throughout the experiment; while the other concentrations and the control group showed more fluctuation. In the control group, the initial oxygen consumption (hour 1) was highest. However, this was not true in any of the groups exposed to Treflan.

Figures 6 and 7 show the changes which take place as time of exposure increases. Plots of oxygen consumption per gram per hour against Treflan concentrations for hours 1 and 2 (Fig. 6) show one pattern of response, with a slightly higher oxygen consumption during hour 2. The plots for hours 3 and 4 (Fig. 7) reveal a different pattern. Initially, hour 5 follows the same pattern as hours 1 and 2, with a decrease in consumption as the Treflan concentration increases from 0 to 0.32 ppm; and an increase in consumption as the Treflan increases from 0.32 to 0.56 ppm (Fig. 6). As the concentration of Treflan increases from 0.56 to 1.0 to 2.0 ppm, however, the pattern of response for hour 5 resembles that in hours 3 and 4 (Fig. 7).

As shown in Figure 8, the mean water temperature and mean oxygen

consumption fluctuations decreased as the Treflan concentration increased.

When the fish are divided into four weight groups, other relationships are revealed. Comparisons of oxygen consumption during hours 1 and 5 show certain trends. At 0 ppm Treflan, the consumption in all weight groups is lower during hour 5, with the greatest decrease in the higher weight group (Fig. 9). The fish at 0.32 ppm (Fig. 10) showed a similar trend. However, at 0.56, 1.0 and 2.0 ppm (Figs. 11-13), the fish in the highest weight group consumed more oxygen per gram during hour 5 than during hour 1. Similarly at 1.0 and 2.0 ppm, the second highest weight group consumed more oxygen during hour 5. In the control group and at 0.32 ppm, the consumption in both hours follows the trend of decreasing oxygen consumption per gram as the weight of the fish increases. However, this trend is altered in the higher concentrations.

The observed behavior of the fish at the higher concentration of Treflan (1.0 and 2.0 ppm) was as described by Latvaitis (1972). Individual fish varied as to times of reaction, but many showed irritable behavior, darkened coloration, and loss of equilibrium. Three of the experimental fish died: one each at 0.32, 1.0, and 2.0 ppm. The deaths were observed at $3\frac{1}{2}$ hours, 4 hours, and $3\frac{1}{2}$ hours, respectively. A few of the fish at lower concentrations also showed irritation and darkening; and many were observed going to the top of the flask (Table 4).

When the oxygen consumption rates were subjected to the Student's t-test, there were no significant differences.

The oxygen consumption for the smallest fish (1.3 grams) tested at 0 ppm Treflan was recorded as .77 ppm per gram during the first hour. This was at least twice the amount of oxygen consumed by any of the other

fish at 0 ppm. It was considered an atypical reaction, so that fish was eliminated from the tabulations. Had it been included, the mean oxygen consumption during the first hour at 0 ppm Treflan would be .25 ppm per gram instead of .20 ppm per gram.

Table 4. Observed behavior of green sunfish (Lepomis cyanellus) over five hours exposure to Treflan E.C.

Treflan concentration (ppm)	Reactions			
	darkening	going to top	loss of equilibrium	dead
0	2	9	0	0
.32	1	5	1	1
.56	1	5	1	0
1.0	4	6	4	1
2.0	5	9	5	1

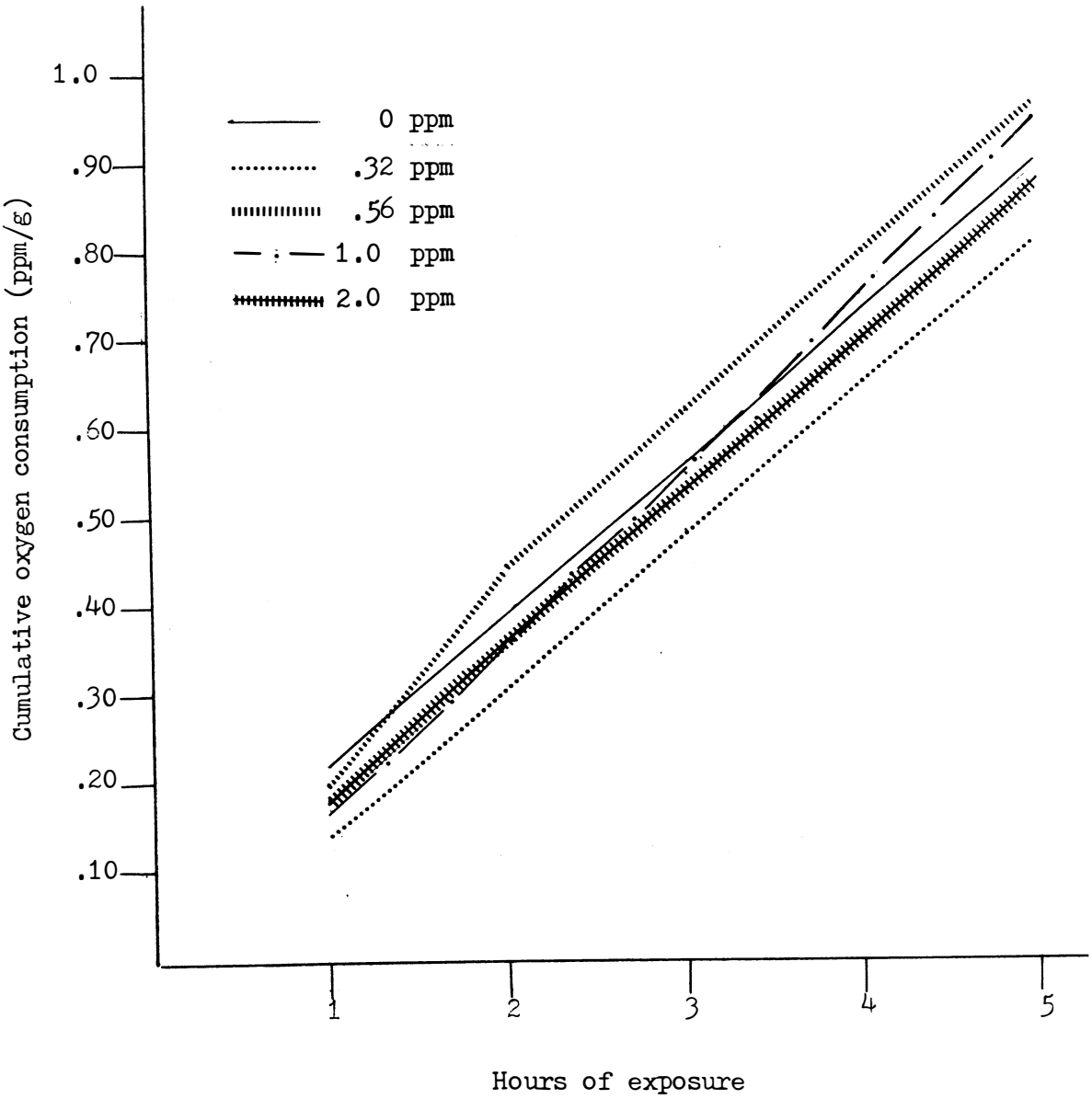

Fig. 4. Cumulative oxygen consumption of green sunfish (Lepomis cyanellus) during five hours exposure to Treflan E.C.

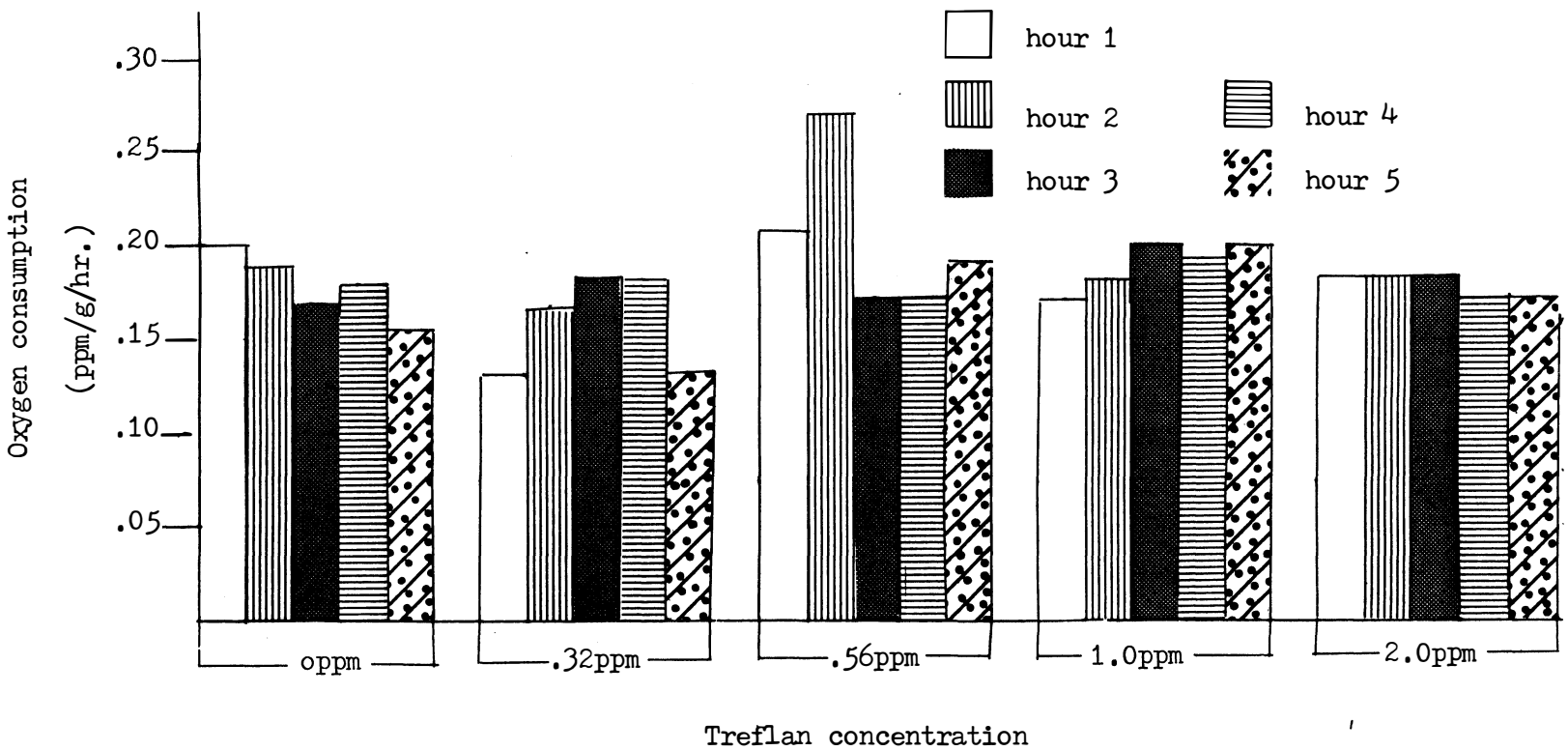

Fig. 5. Oxygen consumption per hour for green sunfish (Lepomis cyanellus) at various Treflan concentrations.

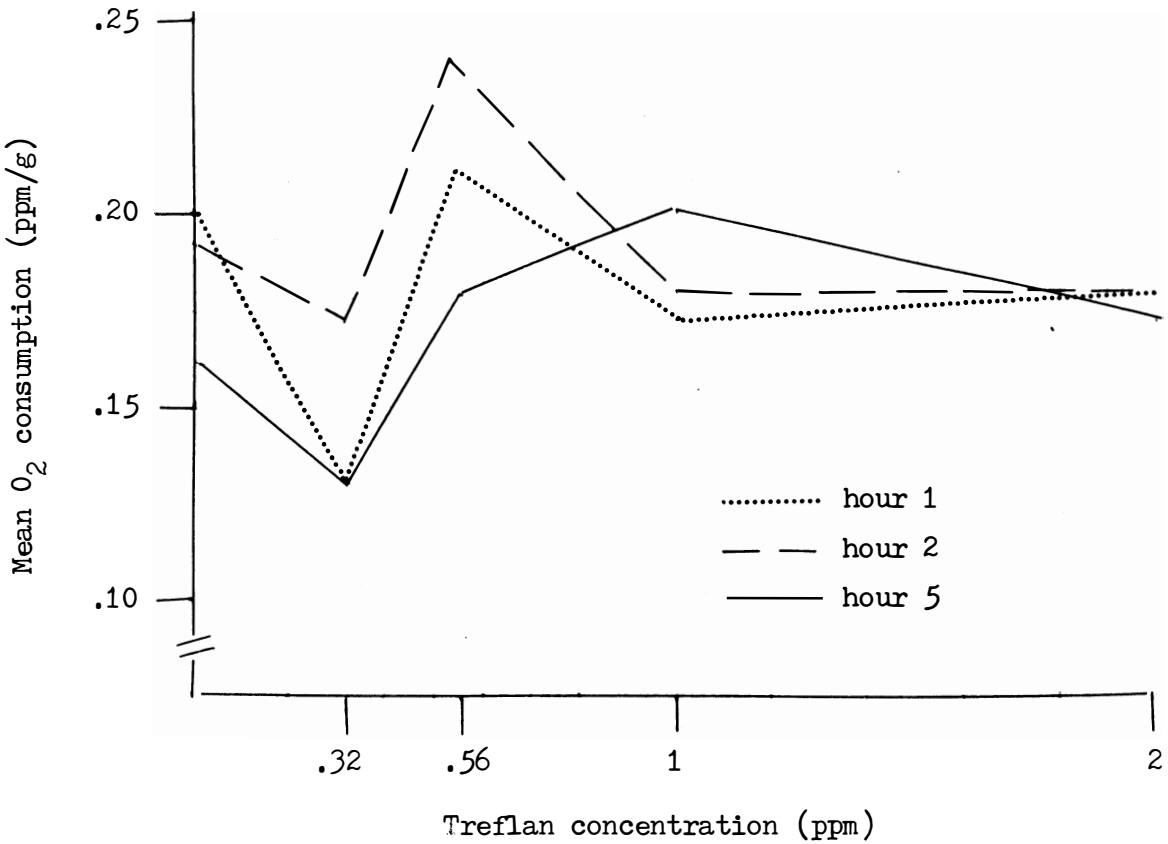

Fig. 6. Oxygen consumption of green sunfish (Lepomis cyanellus) at various Treflan concentrations.

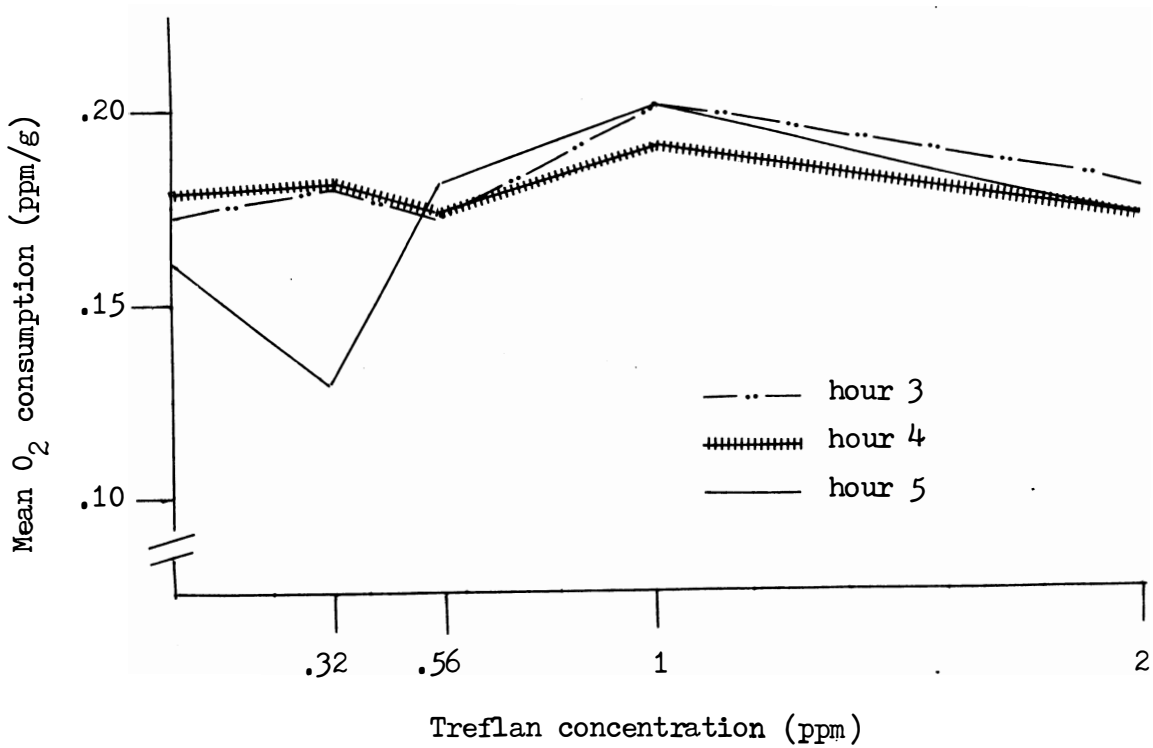

Fig. 7. Oxygen consumption of green sunfish (Lepomis cyanellus) at various Treflan concentrations.

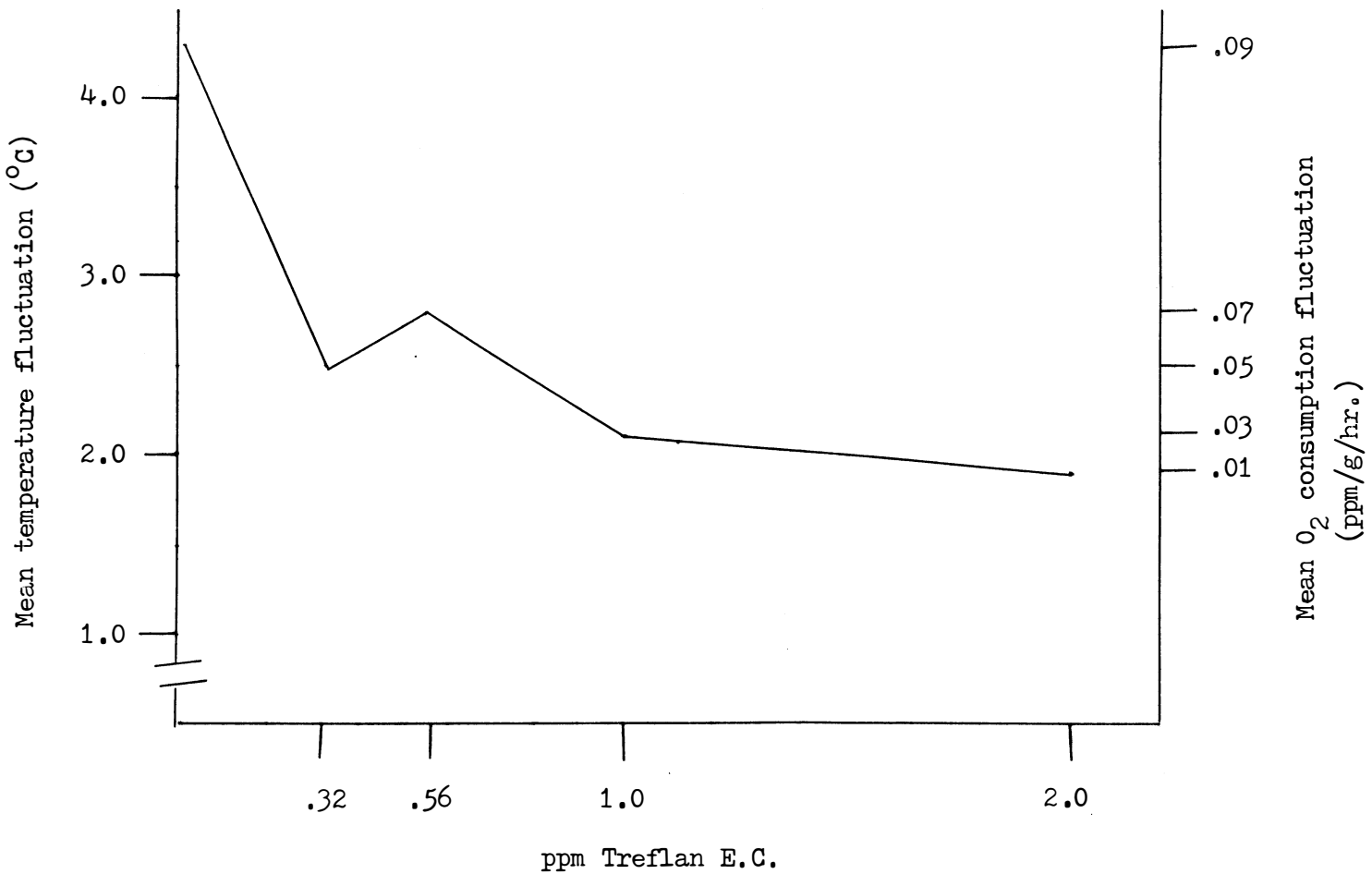

Fig. 8. Mean water temperature & oxygen consumption fluctuations for green sunfish (Lepomis cyanellus) during 5 hours exposure to Treflan E.C.

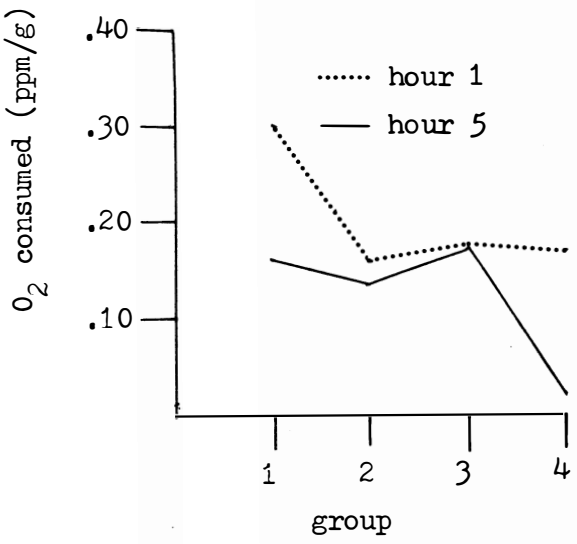

Fig. 9. Oppm

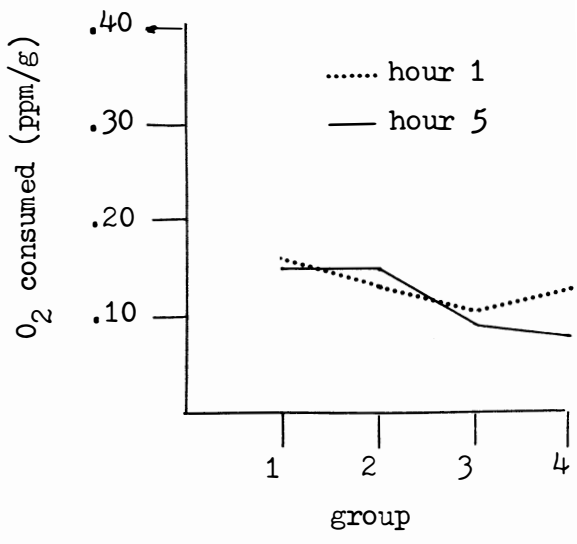

Fig. 10. 0.32ppm

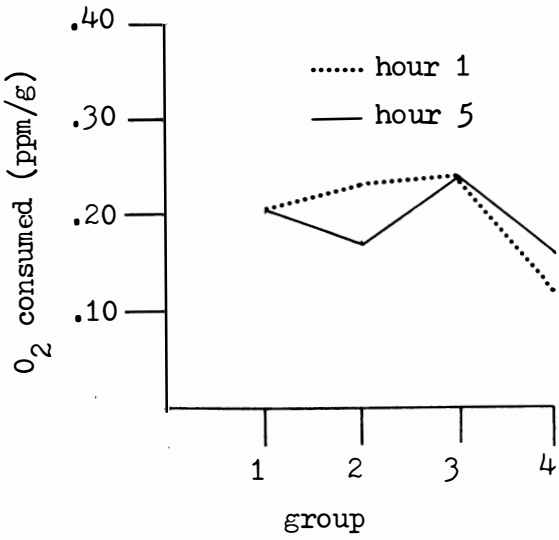

Fig. 11. 0.56ppm

Figs. 9-11. Oxygen consumption of green sunfish (Lepomis cyanellus) during the first and fifth hours of exposure to Treflan E.C.

Group 1 - 1.3 to 2.2 g Group 3 - 3.3 to 4.2 g

Group 2 - 2.3 to 3.2 g Group 4 - 4.3 to 5.3 g

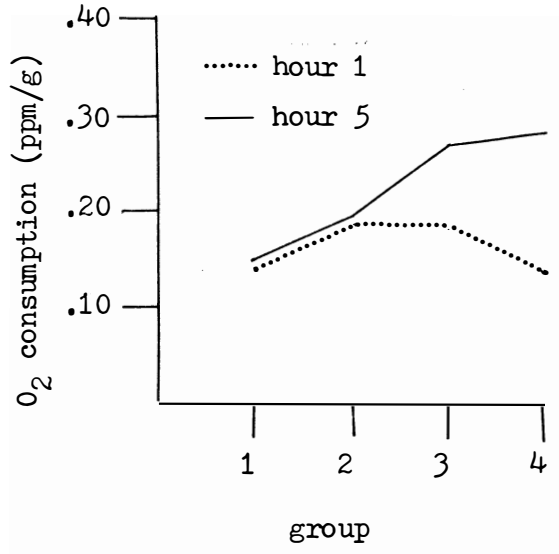

Fig. 12. 1.0ppm

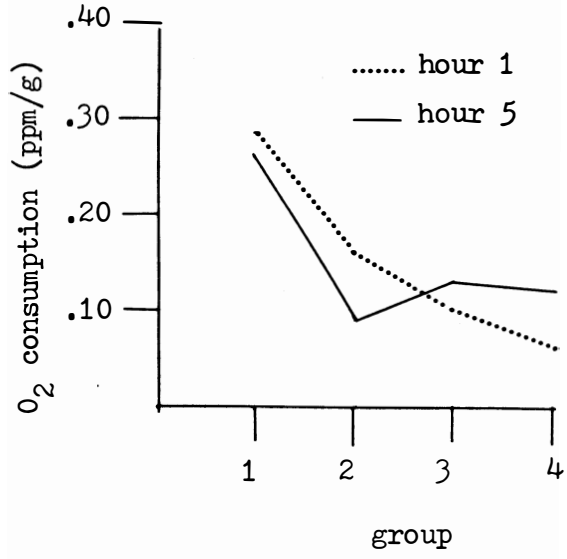

Fig. 13. 2.0ppm

Figs. 12-13. Oxygen consumption of green sunfish (Lepomis cyanellus) during the first and fifth hours of exposure to Treflan E.C.

Group 1 - 1.3 to 2.2 g Group 3 - 3.3 to 4.2 g

Group 2 - 2.3 to 3.2 g Group 4 - 4.3 to 5.3 g

DISCUSSION

Although there was considerable variation in response, the graphs of the mean oxygen consumption rates indicate some trends caused by increasing Treflan concentrations. In comparing the hourly oxygen consumption curves in figures 6 and 7, definite differences can be seen as the time of exposure increases. There is no steady direct relation at all concentrations; but the similarities in the shapes of hours 1 and 2 and in the shapes of hours 3 and 4 appear to confirm their validity. Also interesting in the analysis, is hour 5's similarity to hours 1 and 2 at the lower concentrations and to hours 3 and 4 at the higher concentrations. This indicates that as hour 5 is reached, the fish are becoming acclimated to the lower concentrations (0.32 and 0.56 ppm) and oxygen consumption is returning to the initial values. However, at 1.0 and 2.0 ppm, the fish are not yet acclimated; so the consumption is not returning to normal. Further experiments over longer periods of exposure to Treflan would be desirable. This would determine if there is an adjustment to the higher Treflan concentrations; and if the oxygen consumption rates at the lower concentrations would fluctuate further.

The decrease in the fluctuation of oxygen consumption rates and in the mean temperature fluctuations at 2.0 ppm Treflan (Figs. 5 and 8) suggest a "calming" effect -- or a decrease in metabolic or respiratory changes. A certain amount of fluctuation is expected due to the disturbance of adding the Treflan. Therefore, the lack of fluctuation in oxygen consumption rates at 2.0 ppm is noteworthy.

Fifth hour increases in the oxygen consumption rates of the heavier fish exposed to 0.56, 1.0, and 2.0 ppm Treflan (Figs. 11-13) indicate that Treflan has a greater influence on the larger fish than on the smaller ones. This could be due to physiological changes as the fish matures, greater amounts of Treflan taken into the body or adsorbed onto the gill surfaces, or many other factors, which would require further research.

Although it has been proven toxic to fish, there seems to be little danger of toxic amounts of trifluralin entering natural waters, unless there is improper disposal. Tests conducted using normal application rates show that, regardless of rainfall, the amount of trifluralin present in runoff waters is negligible because of strong adsorption to the soil (Axe et al. 1969, Menges and Tamez 1974). Recent emphasis in toxicity studies has been on acute toxicity -- or what concentration of pollutant will kill 50% of the fish in a short period of time (usually 48 to 96 hours). It is questionable whether this method of determining a safe level of toxicant is valid, since even "negligible" amounts of Treflan present in natural waters could affect fish populations through cumulative effects or "chronic toxicity". This could be via decreased reproductive potential, hyperexcitability or sluggishness, appetite loss, neurological disorders, etc. Also important in this consideration, are the effects on other aquatic organisms which may be present in the food web, and could therefore affect the fish population indirectly.

It seems obvious that Treflan, as well as other herbicides, pesticides, and other artificial means of control should be used very conservatively until much more is known about the specific long-term cumulative effects on aquatic and land organisms.

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