

means of measuring their respective metabolic rates. On a dry weight basis, the slope of the line representing the controls was 1.12, indicating a metabolic rate proportional to weight whereas the slope representing the cyanide-poisoned group was 0.67 indicating a metabolism proportional to surface area.

The size and activity of the fish tested were two factors affecting metabolic rate which was in turn a modifier of cyanide toxicity.

## ACKNOWLEDGEMENTS

This project was supported by a research contract (OSU5-0010) awarded to Dr. Gérard Leduc by the Department of Indian and Northern Affairs, Ottawa.

I would like to extend my sincere thanks to Dr. Gérard Leduc, Associate Professor of Biology at Concordia University, for his help in planning this research, in the analysis of the data and in the preparation of this thesis.

I would also like to thank Dr. Perry Anderson, Assistant Professor of Biology at Concordia University, for his helpful suggestions and constructive criticism of this work.

Dr. Sylvia Ruby, Assistant Professor of Biology at Concordia University, deserves thanks as well for her valuable criticism of this study.

Thanks are also due to Mr. K. Callaghan, Mr. J. Loustau and Mr. K. Cunningham for the technical aid they provided.

The valuable advice and help of fellow graduate students Michel Gaudet, Sam Cheng, Walter Banas, Tibor Kovacs and Diane Galley is gratefully acknowledged.

The support of my parents and Mr. and Mrs. A.E.D. Elliott is sincerely appreciated.

Lastly, I would like to take this opportunity to

state that the understanding, co-operation and generous help of my wife, Katherine Lois McCracken, is greatly appreciated and that her most valuable assistance contributed immeasurably to the completion of this thesis.

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## INTRODUCTION.

The purpose of this laboratory study was to measure the effects of cyanide on the growth of continuously swimming rainbow trout, Salmo gairdneri Richardson, testing the effect of varying ration and of different initial sizes of the fish held in a flow-through system.

Cyanides are used in many manufacturing processes by metal-finishing and mining operations, petroleum refineries, steel mills and other industries. Although examples of gross pollution with cyanides have been diminished by improved waste water controls, chronic pollution occurring by means of continuous release of these cyanides at low concentrations is still very much in evidence (Doudoroff, 1976. p.5). Bérubé and Gilbert (1971) found concentrations of cyanide of  $0.10\text{mg}\cdot\text{l}^{-1}$  at the mouth of a river discharging effluent into Great Slave Lake. In natural surface waters with a normal pH almost all of the free cyanide ( $\text{CN}^-$  and  $\text{HCN}$ ) is in the form of hydrocyanic acid ( $\text{HCN}$ ), which is the most toxic form (Jones, 1964. p.86).

The acute toxicity of cyanide to fish has been shown to be related to temperature. At high, rapidly lethal concentrations of cyanide fish succumb much faster at elevated temperatures (Sumner and Doudoroff, 1938; Anon., 1972). Recently however, Kovacs (1978) has demonstrated that at relatively low, slowly lethal concentrations,

cyanide was more toxic to rainbow trout at lower temperatures; which is in agreement with the findings of The Ministry of Technology, Great Britain (1969). He discovered that the 96-hr. LC50 values at 6, 12 and 18°C were 0.028, 0.042 and 0.068mg·l<sup>-1</sup> HCN respectively.

Some of the adverse effects of chronic cyanide poisoning on fish reported in the literature include the following:

- the impairment of swimming ability in brook trout, Salvelinus fontinalis, (Neil, 1957), coho salmon, Oncorhynchus kisutch, (Broderius, 1970); rainbow trout, Salmo gairdneri, (Speyer, 1975), and cichlids, Cichlasoma bimaculatum, (Leduc, 1966),
- the disruption of iono- and osmoregulatory capacity in rainbow trout (Leduc & Chan, 1971),
- the prevention of maturation of testicular germ cells in rainbow trout (Ruby & Dixon, 1974),
- the impairment of embryological development of Atlantic salmon, Salmo salar Linnaeus (Leduc, 1978),
- reduction in growth rate, disturbance of metabolic rate and necrobiosis of liver hepatocytes in rainbow trout (Dixon, 1975; Speyer, 1975),
- the impairment of yolk deposition in rainbow trout ovaries (Lesniak, 1977).



For a complete review of the toxicity of cyanide to aquatic organisms the reader is referred to Doudoroff (1976).

Growth defined by Warren (1971) as "the formation of tissues from the materials of digested food", is dependent on the metabolic rate of the organism, the energy necessary for maintenance and activity, the quality and quantity of the food consumed and the age or size of the animal itself. In addition, sublethal concentrations of toxicants can reduce the scope for growth by adversely affecting biosynthetic processes or some other vital functions which govern the ability of the animal to assimilate food. Since growth is an integrated result of several biochemical and physiological processes in the animal, it is often used in toxicology work to evaluate the overall capacity of the fish to function in the presence of a toxicant.

In nature, restriction of food supply is the rule rather than the exception (Wurtsbaugh and Davis, 1977a). Yet, there are only a few toxicology studies carried out to measure the effect of varying restricted rations. Oladimeji (1973) fed an artificial diet containing methoxychlor (producing an intake level of 0.67mg/kg/day) to brook trout, Salvelinus fontinalis at rates of 0.5, 1.0, 1.5 and 2.0% of their wet weight per day and found that low feeding rates enhanced the deleterious effect of methoxychlor on growth by markedly increasing the food

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maintenance requirement.

Dieldrin exposure at a concentration of 0.05ppb in the ambient environment severely limited the growth of sculpins, Cottus perplexus, even when food was unrestricted, by increasing the amount of food necessary for maintenance while simultaneously reducing food consumption (Warren, 1971. p.163).

Cichlids subjected to a concentration of  $0.2\text{mg}\cdot\text{l}^{-1}$  of potassium pentachlorophenate while on an unrestricted ration managed to grow as well as the controls by consuming more food, thereby compensating for the decreased efficiency of energy utilization. However, when the food was restricted the poisoned fishes could not compensate and as a consequence they grew much less than the controls (Warren, 1971. p.163).

The effect of cyanide on the growth of fishes fed restricted and unrestricted rations has been examined in earlier studies. Both Speyer (1975) and Dixon (1975) found that juvenile rainbow trout exposed to cyanide while on a restricted ration had significantly reduced growth relative to the control fish.

Leduc (1966) determined that juvenile coho salmon subjected to concentrations of 0.01, 0.02, 0.04 and  $0.08\text{mg}\cdot\text{l}^{-1}$  HCN and fed an unrestricted diet of earthworms showed significantly reduced growth only at the highest concentration. In growth experiments with cichlids exposed to

concentrations of from 0.008 to  $0.10\text{mg}\cdot\text{l}^{-1}$  HCN while on an unrestricted diet of tubificid worms, Leduc (1966) discovered that the cyanide-poisoned fish grew as well as the controls. Hence, in both experiments, Leduc (1966) observed that the unrestricted diet allowing the cyanide-exposed fish to consume more food, permitted them to grow as well as the control fish. The coho salmon also displayed a behavioural adaptation to cyanide poisoning by reducing their activity, thereby lowering their food maintenance requirement and contributing to higher food conversion rates.

In this study, it was decided to feed the fish a series of different restricted rations while being exposed to cyanide, in order to get a more realistic idea of the effect of cyanide in nature where food is not available in unrestricted or relatively large fixed amounts. Also, to prevent any behavioural adaptation to cyanide as experienced by Leduc (1966) the fish were made to swim against a current of fixed velocity, thus ensuring that both the control and cyanide toxified groups maintained the same level of activity.

The toxicity of a pollutant at sublethal concentrations can thus be overcome, in some cases at least partially, by the exposed animal consuming more food. However, a toxic substance may not exert its deleterious effect to the same degree on all sizes of the same species. In acute toxicity tests using pumpkinseed sunfish, Lepomis gibbosus, Spear and

Anderson (1975) discovered a progressive unproportional increase in tolerance to copper poisoning with an increase in size of fish. Anderson and Weber (1975) using guppies, Poecilia reticulata, in a lethal response study found a progressive increase in susceptibility to dieldrin with increase in age class from newborn to adult. Using rainbow trout of different sizes exposed to an acute concentration of 0.153ppm HCN, Hebert and Merkens (1952) determined that with increasing size there was a corresponding progressive decrease in survival time.

Hence, it was decided to test the hypothesis that the toxicity of cyanide was size-dependent at sublethal as well as lethal concentrations. To achieve this, two avenues of research were undertaken; one was to test the effect of activity on the same size of fish, the other was to test different sizes of fish at a uniform swimming speed.

MATERIAL, APPARATUS & METHODS

MATERIAL

The fish used in this study were juvenile rainbow trout, Salmo gairdneri Richardson. It was impossible to obtain fish from the same hatchery for all experiments. Fish used in Experiment 1 came from Pisciculture C.A. Morrissette, Brownsburg, Quebec, those in Experiments 2 and 3 from Pisciculture Truitco, Huntingdon, Quebec, while those in Experiments 4 and 5 came from Pisciculture Mont Sutton, Sutton, Quebec. In all cases, the fish were transported in plastic bags pressurized with oxygen.

Upon arrival at the laboratory at Sir George Williams campus, where this study was carried out, the fish were held in 200 litre, oval fibreglass tanks, each having a continually renewed water supply. The temperature of the water was maintained at  $10 \pm 0.5^{\circ}\text{C}$  and a 12-hour photoperiod, identical to the one used in the experiments, was controlled by a time-switch. The fish were fed an ad libitum ration of Ewos Trout Chow No. 3 on a daily basis throughout the holding period, which lasted 2 - 4 weeks. The fish were apparently healthy and very few mortalities were observed.

APPARATUS

Test Tanks

All experiments in this study of the effects of

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cyanide on the growth of rainbow trout fed different rations and on the growth of trout of different sizes, were performed on fish continually swimming against a current. To achieve this, six annular growth chambers equipped with electric motor-driven paddlewheels producing currents ranging from 0 - 20.4 cm·sec<sup>-1</sup> were used (Kruzynski, 1972). Each 90 litre fibreglass tank was circular in shape having a diameter of about 102 cm., a depth of 21 cm. and a width of 23 cm. To prevent the fish from jumping out, the top of each tank was covered with fibreglass mosquito screening. Manostat-predictability flowmeters, (Manostat Corp., New York, N.Y.), maintained a constant flow of water into each tank at 1000 ml·min<sup>-1</sup>. For the largest group of fish employed, 1.8 litres of test water per gram of fish per day was provided with 90% replacement occurring in 3.5 hours, thus meeting the limits recommended by Sprague (1973). The stock solutions of cyanide were metered into the growth chambers using a Manostat Cassette Pump (Manostat Corp., New York, N.Y.). Two shock rings in each tank, producing a current of 3.15 volts AC, served to prevent the fish from resting behind the standpipe and to discourage them from drifting with the current.

Every tank was illuminated by a 40-watt lightbulb and a time-switch controlled the 12-hour photoperiod. The entire assembly, illustrated in Figures 1 and 2, was covered completely with black plastic sheeting to minimize

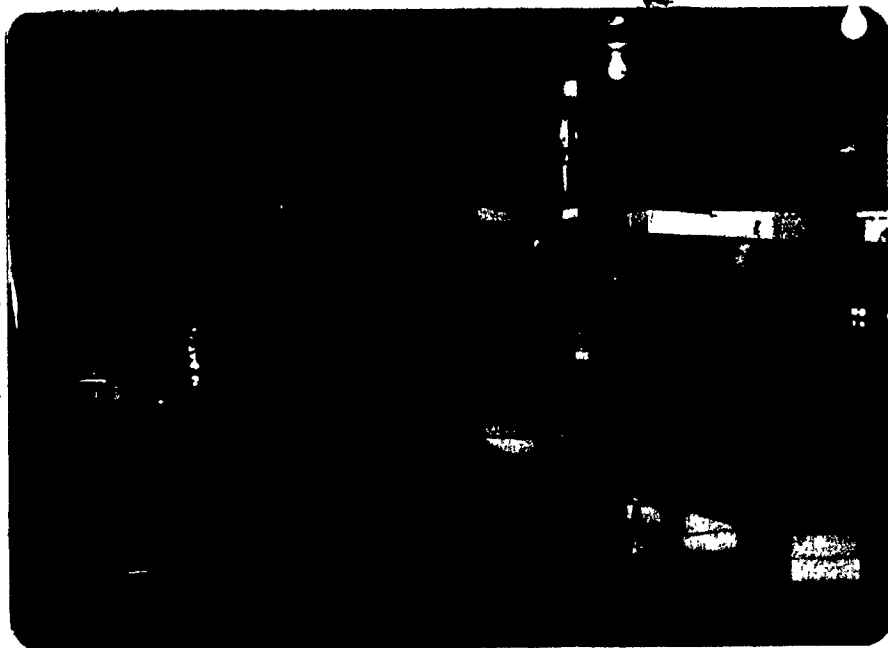
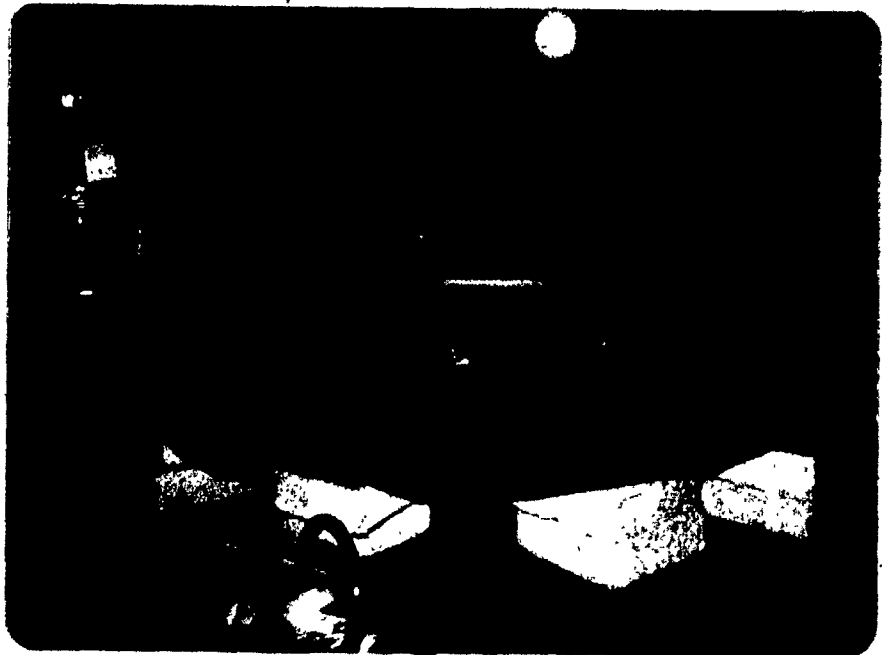


Figure 1. Photograph of the experimental assembly showing four of the six annular growth chambers used to continuously expose juvenile rainbow trout to hydrogen cyanide which was metered into the tanks from the stock solutions using a peristaltic pump.

*coloured picture*



•Figure 2. Photograph of one annular growth chamber in which juvenile rainbow trout were held, swimming against a constant water velocity while being continuously exposed to hydrogen cyanide.

*coloured picture*



disturbance to the fish.

Water Supply

A constant supply of water at  $10 \pm 0.5^{\circ}\text{C}$ , available throughout the year, was used in all five experiments. Charcoal filters dechlorinated the water from the City of Montreal and plastic (PVC) piping delivered it to the experimental apparatus.

The chemistry of the water during the experimental period, presented in Table 1, was obtained from the City of Montreal Public Works Department.

Table 1: Chemical analysis of treated water for the City of Montreal from January 1975 to January 1976.

Alkalinity $\text{CaCO}_3$ ( $\text{mg}\cdot\text{l}^{-1}$ )	Total Hardness ( $\text{mg}\cdot\text{l}^{-1}$ )	$\text{CO}_2$ ( $\text{mg}\cdot\text{l}^{-1}$ )	pH
86	126	0.5	7.9

With a decrease in pH there is a consequent increase in the toxic form of free cyanide, hydrocyanic acid (HCN). Jones (1964, p. 87) cites evidence that in a pH range of 7.4 to 8.0 that at least 93% of the free cyanide would be in

the form of undissociated HCN. Hence, in these experiments with a pH of 7.9, the fish were exposed almost entirely to hydrocyanic acid.

Dissolved oxygen concentrations in the growth chambers, determined by the Winkler Method - Azide Modification (Standard Methods, 1971), ranged from 8.1 to 10.7 mg  $\cdot$  l<sup>-1</sup> or an average of 84% saturation.

## METHODS

### Diet

The diet used in all five experiments was dry pelleted formula, Ewos Trout Chow No. 3. It was screened through a U.S. Standard Sieve (ASTM E:11 Mesh No. 50) to remove the powdered food that the fish would be unable to consume. The food was then dried in an oven at 70°C for 1 week to eliminate the water content, prior to being stored in sealed containers. The components of the diet are listed below:

Protein	49.5%
Fat	9.7
Carbohydrate	28.3
Ash	9.7
Fibre	2.8

### Handling of Fish

In this study of the effects of cyanide on the growth of rainbow trout fed different rations and on

different sizes of rainbow trout, each of the five experiments was 20 days in length.

In preparation for an experiment, the trout were selected on the basis of wet weight, after anesthetizing them in a solution containing  $20\text{mg}\cdot\text{l}^{-1}$  MS 222 (tricaine methane sulphate), blotting them dry and weighing each individual to the nearest hundredth of a gram. They were then introduced into each of the six test tanks, 37 fish/tank, using a table of random numbers, and held for 2 weeks on a ration of  $2.0\%\cdot\text{day}^{-1}$  before Experiments 1, 2 and 3. For experiments 4 and 5, they were acclimatized to the test conditions at a ration of  $1.0\%\cdot\text{day}^{-1}$ . Only 30 fish/tank were to be used in the experiment but additional fish were required to account for any mortality that might occur during the holding period and also so that a pre-experimental sample could be taken.

The fish were then starved for 24 hours at the end of the holding period, removed, anesthetized with MS 222, weighed and individually marked with liquid nitrogen using the method of Mighell (1969) before being returned to their respective tanks. A group of fish randomly selected and sacrificed, served as a pre-experimental sample and was frozen for later analysis of body constituents. The stock cyanide solutions, made up as described by Leduc (1966), were metered into 3 of the 6 growth chambers the next day, marking the beginning of the experiment. Each control and

cyanide-exposed group of fish was fed a daily restricted<sup>D</sup> ration expressed as percent dry weight of food/wet weight of fish. The trout were starved for a period of 24 hours before each weighing. On day 10, the fish were removed, anesthetized and weighed as previously described, returned to their respective tanks and had their ration levels adjusted accordingly. The experiment was terminated on day 20 whereupon the fish were removed, weighed and sacrificed. They were then placed in a drying oven, along with the pre-experimental sample, for 7 days at 70°C in order to determine the wet weight dry weight ratios.

The members of each group were subsequently pooled together and ground up in a Waring blender; each sample was then stored in a separate sealed container prior to a fat content determination being carried out using a Goldfisch Fat Extractor (Model 35003). Ether was used as a solvent on a 2.0g sample of dry whole fish tissue subjected to a 4-hour reflux distillation.

Routine daily maintenance consisted of cleaning the tanks prior to feeding, as well as monitoring the diluent water and cyanide flow rates. Dissolved oxygen determinations using the Winkler Method - Azide Modification (Standard Methods, 1971) were carried out on a daily basis. Cyanide determinations were done on alternate days using Epstein's colorimetric method (Standard Methods, 1971) in Experiment 1 and the method of Lambert et al. (1975) in

subsequent experiments. The observed values never varied by more than 5% from the predicted ones.

All experiments had been planned to be carried out at  $0.010\text{mg}\cdot\text{l}^{-1}$  HCN. Unfortunately, in Experiment 1, it was not noticed until the experiment was over that due to an error in calculations the cyanide concentration used was  $0.013\text{mg}\cdot\text{l}^{-1}$  HCN instead of  $0.010\text{mg}\cdot\text{l}^{-1}$  HCN. A list of conditions under which each experiment was conducted is presented in Table 2.

Table 2: Cyanide concentrations, rations, swimming speeds, initial mean wet weights, initial mean lengths and the number of fish beginning and ending each 20-day experiment carried out on rainbow trout at 10°C

Expt. No.	HCN (mg.l <sup>-1</sup> )	Ration (%.day <sup>-1</sup> )	Swimming Speed (cm.sec <sup>-1</sup> )	Initial Mean Wet Weight (grams)	Initial Mean Length (cm.)	No. of Fish at Beginning of Expt.	No. of Fish at End of Expt.
1	0.000	0.5	12.1	18.67	11.7	30	28
	0.013	0.5	12.1	18.38	11.6	30	29
	0.000	1.0	12.1	20.41	11.8	30	29
	0.013	1.0	12.1	19.05	11.7	30	28
	0.000	1.5	12.1	19.09	11.7	30	27
	0.013	1.5	12.1	17.90	11.7	30	28
2	0.000	0.0	12.1	8.02	8.5	30	29
	0.010	0.0	12.1	7.81	8.3	30	28
	0.000	1.0	12.1	8.40	8.6	30	30
	0.010	1.0	12.1	8.08	8.4	30	29
	0.000	2.0	12.1	7.88	8.4	30	30
	0.010	2.0	12.1	8.00	8.5	30	25
3	0.000	0.0	12.1	7.87	8.7	30	28
	0.010	0.0	12.1	8.23	8.7	30	28
	0.000	1.0	12.1	7.61	8.6	30	30
	0.010	1.0	12.1	7.95	8.7	30	29
	0.000	2.0	12.1	7.74	8.6	30	30
	0.010	2.0	12.1	7.91	8.7	30	30
4	0.000	1.0	6.7	11.66	10.0	30	30
	0.010	1.0	6.7	11.88	10.1	30	30
	0.000	1.0	12.1	11.59	10.0	30	30
	0.010	1.0	12.1	10.96	9.9	30	29
	0.000	1.0	20.4	11.23	9.9	30	28
	0.010	1.0	20.4	11.33	9.9	30	30
5	0.000	1.0	12.1	5.56	7.8	30	29
	0.010	1.0	12.1	5.78	8.0	30	29
	0.000	1.0	12.1	16.92	11.3	30	30
	0.010	1.0	12.1	16.00	11.0	30	29
	0.000	1.0	12.1	26.86	13.2	30	29
	0.010	1.0	12.1	27.60	13.4	30	29

### Experimental Design

This study was initially planned to measure the effect of cyanide on the food maintenance requirement of rainbow trout held in swimming chambers while being fed different rations of food. In Experiments 1, 2 and 3 conducted with a current of  $12.1\text{cm}\cdot\text{sec}^{-1}$ , the initial average wet weights of the fish were 18.92, 8.01 and 7.89g respectively. Each pair of control and cyanide-exposed fish was fed a different ration; the rations being 0.5, 1.0 and  $1.5\%\cdot\text{day}^{-1}$  in the first experiment and 0.0, 1.0 and  $2.0\%\cdot\text{day}^{-1}$  in Experiments 2 and 3. The pre-experimental samples in each of these experiments consisted of 20 trout.

Upon analysis of the results obtained in Experiments 1, 2 and 3 there seemed to be a cyanide size-related effect. To ascertain this, two approaches were taken; one was to measure the effect of activity on uniform size fish while the other was to measure the effect of the initial size at a uniform swimming speed. To determine whether the toxicity of cyanide could be modified by the activity of the fish, three different swimming speeds of 6.7, 12.1 and  $20.4\text{cm}\cdot\text{sec}^{-1}$  were established and tested in Experiment 4. The ration level of each group was  $1.0\%\cdot\text{day}^{-1}$  and the initial average wet weight of the fish was 11.44g. The pre-experimental sample was comprised of 3 groups of 10 fish randomly taken from tanks having the same current velocity.

The effect of initial size on the toxicity of chronic cyanide poisoning was tested in Experiment 5 on groups of fish having initial average wet weights of 5.67, 16.46 and 27.23g. All test groups swam against a current of  $12.1\text{cm}\cdot\text{sec}^{-1}$ , as in Experiments 1, 2 and 3 and were given a ration of  $1.0\%\cdot\text{day}^{-1}$ . Each size group had 10 randomly selected fish as a pre-experimental sample.



## RESULTS

The fish held their position in the current and readily accepted the test diet during the acclimation period. No difference in behaviour between the control and cyanide-exposed fish was discernable in any of the growth chambers after the introduction of cyanide.

The formula for Average Relative Growth Rate was used to determine the changes in wet, dry and fat weight of the test fish in each 20-day experiment since Warren (1971, p.139) has indicated it to be appropriate when measuring the growth of an animal over a short period of time. The results were calculated with the formula given below, from the individual data obtained from each individually marked fish.

$$GR = \frac{W_2 - W_1}{0.5(W_1 + W_2)(t_2 - t_1)}$$

Where:

- GR = Average Relative Growth Rate ( $\text{mg} \cdot \text{g}^{-1} \cdot \text{day}^{-1}$ )
- $W_2 - W_1$  = Change in weight from day 0 to day 20 (mg)
- $0.5(W_1 + W_2)$  = Average weight over 20-day interval (g)
- $(t_2 - t_1)$  = Sampling interval (20 days)

## Effects of different rations

The average relative growth rates based on wet weight changes in Experiments 1, 2 and 3 are given in Table 3 and illustrated in Figure 3. They indicate that in Experiment 1 the 18g fish exposed to a concentration of

Table 3. Average Relative Growth Rates based on wet and dry weights and fat content of rainbow trout exposed to various concentrations of cyanide and held on a series of rations while swimming against a current of 12.1 cm-sec<sup>-1</sup> in continuously renewed water at 10°C.

Expt. No.	Cyanide Conc. (mg·l <sup>-1</sup> )	Ration %·day <sup>-1</sup>	Initial Mean Met Weight (g)	Final Mean Met Weight (g)	Average Relative Growth Rate (Met Weight) (mg·g <sup>-1</sup> ·day <sup>-1</sup> )	Average Relative Growth Rate (Dry Weight) (mg·g <sup>-1</sup> ·day <sup>-1</sup> )	Average Relative Growth Rate (Fat Content) (mg·g <sup>-1</sup> ·day <sup>-1</sup> )	% Value for Comparison of Growth of Control vs Cyanide-Toxified Group (Met Weight)
1	0.000	0.5	18.67	19.37	1.94	0.53	-7.69	-
	0.013	0.5	18.38	18.49	-0.32	-9.31	-20.01	3.10**
	0.000	1.0	20.41	23.11	6.18	6.59	2.79	-
	0.013	1.0	19.05	19.53	1.80	0.13	-10.11	4.29***
	0.000	1.5	19.09	22.49	8.35	7.85	2.30	-
0.013	1.5	17.90	18.62	1.34	-1.20	-15.76	5.56***	
2	0.000	0.0	8.02	6.71	-9.07	-12.84	-22.02	-
	0.010	0.0	7.81	6.42	-9.85	-13.17	-21.50	1.37
	0.000	1.0	8.40	8.87	2.37	2.60	6.16	-
	0.010	1.0	8.08	8.83	3.61	4.90	9.62	-1.14
	0.000	2.0	7.88	9.41	8.33	10.22	19.44	-
0.010	2.0	8.00	9.15	6.78	7.74	16.75	-	
3	0.000	0.0	7.87	6.80	-8.90	-11.84	-18.83	-
	0.010	0.0	8.23	6.75	-9.88	-13.21	-21.10	2.28*
	0.000	1.0	7.61	8.16	3.13	3.80	10.10	-
	0.010	1.0	7.95	8.70	3.78	5.07	11.25	-0.92
	0.000	2.0	7.74	9.60	9.97	12.90	22.46	-
0.010	2.0	7.81	9.79	10.09	13.06	23.21	-	

\* p < 0.05  
 \*\* p < 0.01  
 \*\*\* p < 0.001

WET WEIGHT GAIN

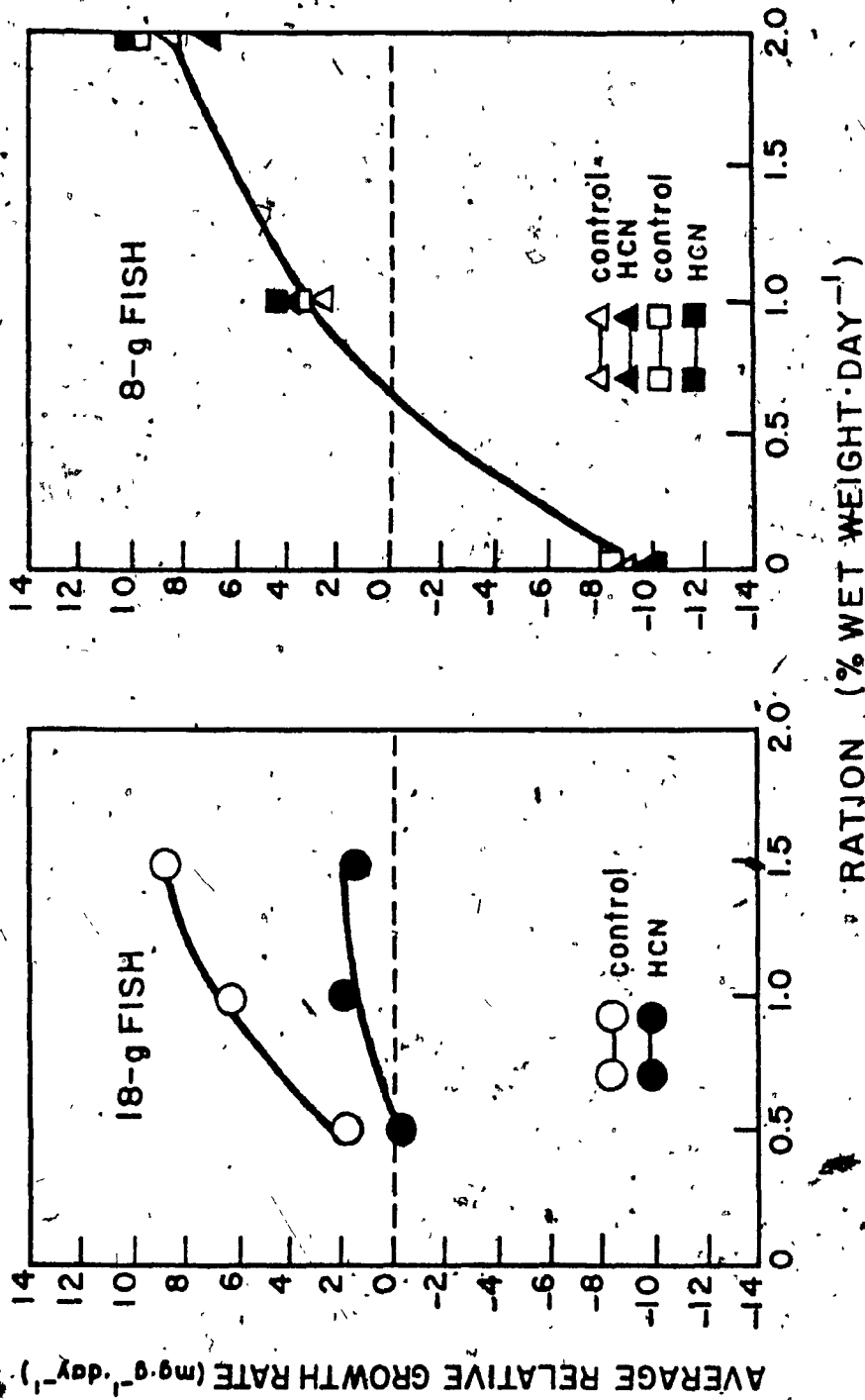


Figure 3. The relationship between the Average Relative Growth Rate based on wet weight changes of 18 and 8g juvenile rainbow trout and the daily food rations received while swimming against a current of 12.1cm·sec<sup>-1</sup> at 10°C, comparing control and cyanide-exposed fish.

$0.013\text{mg}\cdot\text{l}^{-1}$  HCN suffered a severe reduction in growth rate relative to the controls, the effect becoming progressively more pronounced with each increasing ration. In Experiments 2 and 3 however, the results were quite different as there was no obvious effect of cyanide. Using 8g trout subjected to  $0.010\text{mg}\cdot\text{l}^{-1}$  HCN, it was found that the cyanide-exposed fish in both experiments at the zero feeding level exhibited a greater weight loss than the controls. At the 1.0 and 2.0 percent feeding levels, the effect of cyanide on growth was unclear. In some cases the cyanide-toxified fish grew better, in others, they grew less than the controls.

The results of Student's *t* - tests, used in each of Experiment 1, 2 and 3 to statistically compare the control and cyanide-poisoned fish at each ration level, are presented in Table 3. In Experiment 1 there was a highly significant difference between the control and cyanide-exposed fish at every feeding regime tested, confirming the deleterious effect of cyanide on wet weight gain.

At the  $0.0\%\cdot\text{day}^{-1}$  feeding level, a significant difference between the control and toxified groups was demonstrated in Experiment 3, but not in Experiment 2. In neither experiment at the  $1.0\%\cdot\text{day}^{-1}$  ration level was there a significant difference between the control and cyanide-exposed trout. No *t* - tests were conducted for those fish receiving a ration of  $2.0\%\cdot\text{day}^{-1}$  since the control and cyanide-toxified trout in Experiment 3 showed nearly

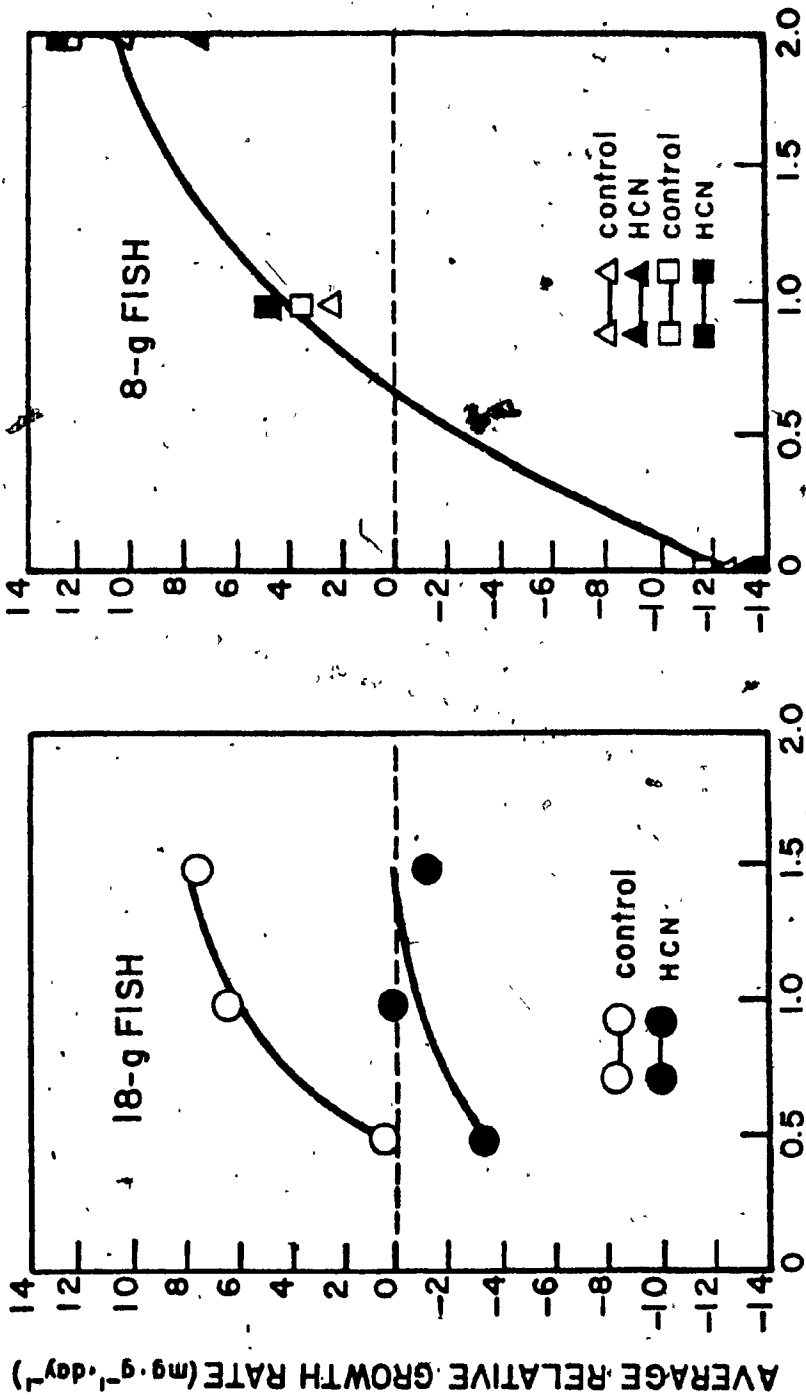
identical growth rates while those in Experiment 2 were similar. Hence, only at the starvation ration in Experiment 3 was there a significant difference between the control and cyanide-poisoned fish.

It is important when assessing the growth of fish to measure dry weight changes as well as those of wet weight, because the water content of the fish can change over time and thus obscure the true rate of growth. Hence, the average relative growth rates based on dry weight were calculated and are given in Table 3 and illustrated in Figure 4.

Both the control and cyanide-exposed trout in Experiment 1 receiving the smallest ration of  $0.5\% \cdot \text{day}^{-1}$ , showed lower dry weight than wet weight growth rates indicating an increase in water content. The controls barely gained any dry weight while the toxified group suffered a drastic loss. At 1.0 and 1.5 percent feeding levels, the control fish had dry weight growth rates similar to their respective wet weight growth rates indicating a true elaboration of tissue whereas the cyanide-poisoned fish, at corresponding feeding levels, had taken up water thereby reducing their growth rates and further magnifying the disparity in growth between the two groups.

In Experiments 2 and 3, both the control and cyanide-toxified fish at the  $0.0\% \cdot \text{day}^{-1}$  ration level exhibited greater losses in dry weight than wet weight reflecting an increase in water content during the course

DRY WEIGHT GAIN



RATION (% WET WEIGHT·DAY<sup>-1</sup>)

Figure 4. The relationship between the Average Relative Growth Rate based on dry weight changes of 18 and 8g juvenile rainbow trout and the daily food rations received while swimming against a current of 12.1cm·sec<sup>-1</sup> at 10°C, comparing control and cyanide-exposed fish.

60

of the experiment. The cyanide-exposed groups in both cases lost more weight than the controls, confirming the similar effect of cyanide previously observed on wet weight (See Figure 3).

At the  $1.0\% \cdot \text{day}^{-1}$  feeding level, the controls experienced dry weight gains similar to their wet weight changes whereas the respective cyanide-exposed groups had higher dry weight growth rates, indicating a loss of water associated with an accelerated growth rate. The fish receiving a ration of  $2.0\% \cdot \text{day}^{-1}$  showed dry weight growth rates greater than those based on wet weight indicating a reduction in water content. In Experiment 2, the control group grew better than the cyanide-poisoned fish while in Experiment 3, the control and toxified groups grew at the same rate thereby supporting the findings of the wet weight analysis.

Because the nutritional well-being of a fish is reflected by its fat content, the changes in fat were computed and are presented in Table 3 and Figure 5. In the first experiment, it can be seen that at the  $0.5\% \cdot \text{day}^{-1}$  feeding regime, the cyanide-exposed fish suffered a loss in fat content nearly 3 times that of the controls. At the 1.0 and 1.5 percent ration levels, the controls gained small similar amounts of fat while the respective toxified groups lost substantial amounts.

In Experiments 2 and 3, both the control and cyanide-

FAT WEIGHT GAIN

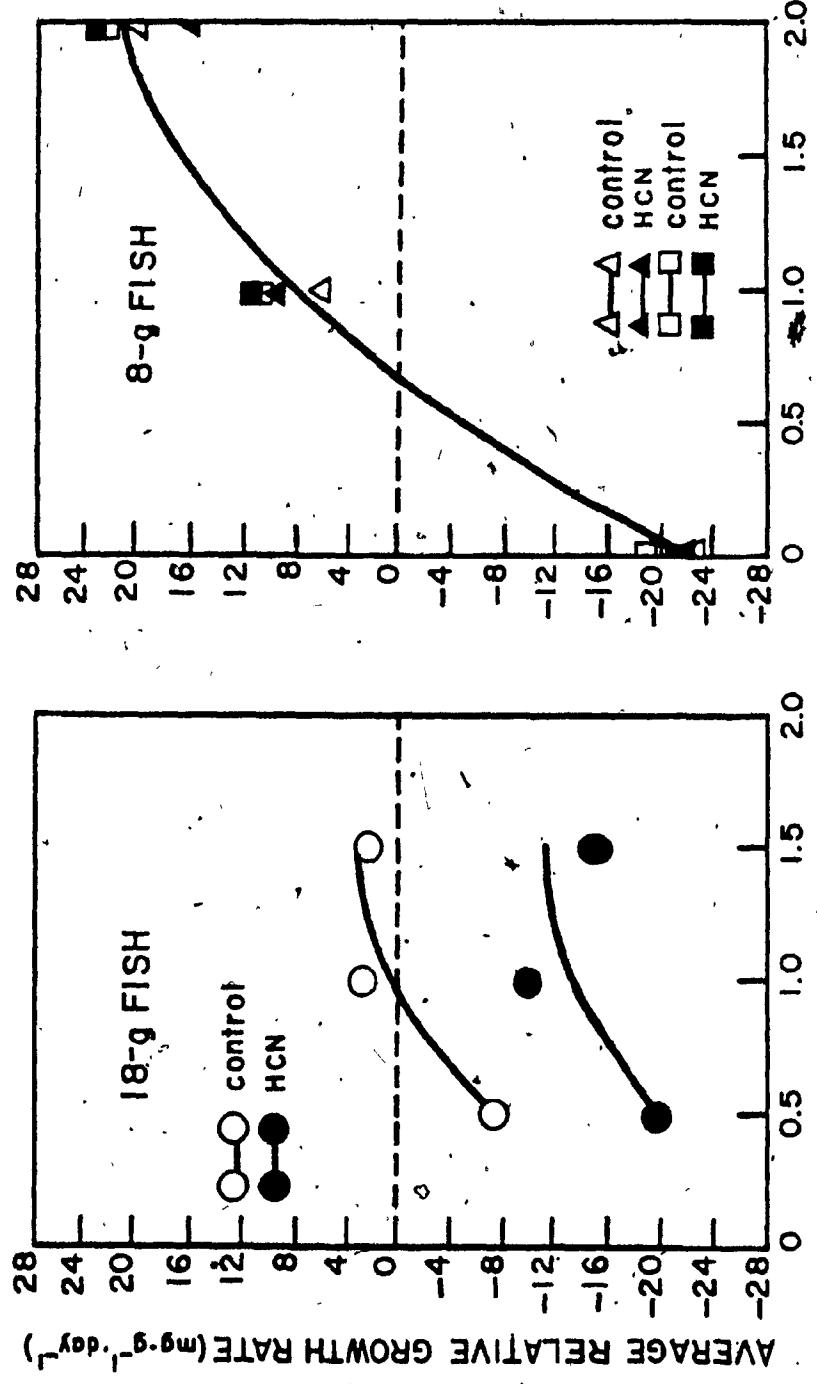


Figure 5. The relationship between the Average Relative Growth Rate based on fat weight changes of 18 and 8g juvenile rainbow trout and the daily food rations received while swimming against a current of 12.1cm·sec<sup>-1</sup> at 100°C, comparing control and cyanide-exposed fish.



poisoned trout at each ration level showed similar changes in fat content. However, it is interesting to note that at the  $1.0\% \cdot \text{day}^{-1}$  feeding level, that both the control and cyanide-exposed fish in all 3 experiments exhibited gains in dry weight that were approximately the same, except for the 18g, cyanide-toxified group in Experiment 1. Yet the 8g fish were adding considerably more fat than the 18g ones.

#### Effects of cyanide on the food maintenance requirement

The average relative growth rates of Experiments 1, 2 and 3 based on wet, dry and fat weight gains of rainbow trout swimming against a current of  $12.1 \text{ cm} \cdot \text{sec}^{-1}$ , graphically illustrated in Figures 3, 4 and 5 were used) to estimate food maintenance requirement (the amount of food required for a fish to neither gain nor lose weight). This is achieved by reading from the graph of growth rate vs. ration, the ration corresponding to the point of zero growth.

From the wet weight growth rates in Figure 3, it is apparent that cyanide has increased the food maintenance requirement of the 18g trout subjected to  $0.013 \text{ mg} \cdot \text{l}^{-1}$  HCN in Experiment 1 from about 0.45 for the controls to  $0.60\% \cdot \text{day}^{-1}$  for the toxified group. However, for the 8g fish subjected to  $0.010 \text{ mg} \cdot \text{l}^{-1}$  HCN in Experiments 2 and 3, the food maintenance requirements was the same,  $0.65\% \cdot \text{day}^{-1}$ , for both the control and cyanide-poisoned groups.

The growth rates based on dry weight depicted in

Figure 4 revealed that the cyanide-toxified fish in Experiment 1 had taken up water, resulting in lower growth rates. Consequently, the food maintenance requirement was increased 2 - 3 times the control level of  $0.50\% \cdot \text{day}^{-1}$ . For the controls and cyanide-exposed fish in Experiments 2 and 3, the amount of food necessary to maintain themselves at zero growth was determined to be  $0.65\% \cdot \text{day}^{-1}$ , the same as that found under wet weight analysis.

From Figure 5 it can be seen that a ration of  $0.95\% \cdot \text{day}^{-1}$  was necessary to maintain a zero growth rate with regard to fat content for the 18g controls, almost double that based on wet and dry weight. The cyanide-poisoned fish suffered reductions in fat levels at all rations tested making it impossible for them to maintain a fat maintenance level. In Experiments 2 and 3, both the control and toxified groups had a food maintenance requirement based on fat content of  $0.65\% \cdot \text{day}^{-1}$ , the same as that determined for wet and dry weight.

#### Effects of activity

The results of Experiments 1, 2 and 3 show clearly that the 18g trout were adversely affected with respect to growth rate in the presence of cyanide whereas the 8g trout were not, except perhaps at the zero feeding level. Part of the increased toxicity experienced by the larger trout could be due to the slightly higher concentration of cyanide

to which they were exposed, but it is doubtful that this could explain the large difference in response between the two groups. Instead, a size effect was suspected. At a uniform swimming speed of  $12.1\text{cm}\cdot\text{sec}^{-1}$  the smaller fish had to expend more energy for swimming than the larger ones. Hence, it was postulated that the higher relative activity and metabolic rate of the smaller trout could be responsible for overcoming the toxic action of cyanide. The results of one experiment using uniform size trout, of about 11g, exposed to a concentration of cyanide of  $0.010\text{mg}\cdot\text{l}^{-1}$  HCN and swimming at 3 different current speeds of 6.7, 12.1 and  $20.4\text{cm}\cdot\text{sec}^{-1}$  are given in Table 4. On the basis of wet weight gain, the control and cyanide-toxified fish displayed almost identical growth rates at the 6.7 and  $12.1\text{cm}\cdot\text{sec}^{-1}$  current velocities while at  $20.4\text{cm}\cdot\text{sec}^{-1}$  the cyanide-poisoned trout were stimulated to grow more than the controls. A statistical analysis by means of Student's t - tests confirmed the lack of any difference between the control and toxified groups at either 6.7 or  $12.1\text{cm}\cdot\text{sec}^{-1}$  but did support the significantly faster growth of the cyanide-exposed fish at  $20.4\text{cm}\cdot\text{sec}^{-1}$ .

The small decrease in dry weight growth rate for the controls at  $6.7\text{cm}\cdot\text{sec}^{-1}$  reflected a marginal increase in water content. The corresponding cyanide-poisoned fish exhibited a nearly identical growth rate based on dry weight as that based on wet weight, thus maintaining the same

Table 4. Average Relative Growth Rates based on wet and dry weights and fat content of rainbow trout exposed to various concentrations of cyanide while swimming against different current speeds and being fed at a rate of 0.05% day<sup>-1</sup> in continuously renewed water at 10°C.

Expt. No.	Cyanide Conc. (mg·l <sup>-1</sup> )	Swimming Speed (cm·sec <sup>-1</sup> )	Initial Mean Wet Weight (g)	Final Mean Wet Weight (g)	Average Relative Growth Rate (Wet Weight) (mg·g <sup>-1</sup> ·day <sup>-1</sup> )	Average Relative Growth Rate (Dry Weight) (mg·g <sup>-1</sup> ·day <sup>-1</sup> )	Average Relative Growth Rate (Fat Content) (mg·g <sup>-1</sup> ·day <sup>-1</sup> )	"t" Value for Comparison of Growth of Control vs Cyanide-Toxified Group (Wet Weight)
4	0.000	6.7	11.66	13.13	5.79	5.09	6.41	0.04
	0.010	6.7	11.88	13.39	5.75	5.85	7.20	-
	0.000	12.1	11.59	12.98	5.45	6.33	10.65	-0.11
	0.010	12.1	10.96	12.12	5.54	6.46	10.19	-
0.000	20.4	11.23	11.80	2.74	3.15	2.62	-1.99*	
0.010	20.4	11.33	12.19	3.47	4.61	5.69	-	

\* p < 0.05

water level throughout the experiment. Both the control and cyanide-exposed trout at the intermediate swimming speed showed a decrease in water content as was indicated by the slightly higher dry weight growth rates. Similarly, at  $20.4\text{cm}\cdot\text{sec}^{-1}$ ; the control and cyanide-toxified groups had substantially increased their respective dry weight growth rates, the latter having grown nearly 1.5 times more than the former.

Considering fat gain, the fish subjected to cyanide at the lowest velocity increased their lipid content slightly more than the controls while both the control and toxified groups swimming at  $12.1\text{cm}\cdot\text{sec}^{-1}$  added fat at the same rate. At the highest velocity tested the cyanide-poisoned fish increased their fat content twice as much as the controls did.

#### Effect of initial size on wet and dry weight gain

The results of Experiment 4 confirmed those of Experiments 2 and 3, that cyanide had little effect on the inhibition of growth of 11g trout at low levels of activity. The highest level of activity accompanied by the highest metabolic rate did however accelerate the growth of the fish subjected to cyanide, suggesting that it was possible to influence the toxicity of cyanide by altering the metabolic rate. It was thought then that the tremendous difference in response observed between Experiments 1 and 2 and 3 was largely due to the difference in metabolic rate between the

8 and 18g trout. The hypothesis was put forward that since the weight specific metabolic rate of smaller fish is higher than that of larger fish (Winberg, 1956), that the smaller fish would be able to overcome cyanide poisoning and grow as well as or better than the controls whereas the larger fish could not and consequently would show a reduction in growth when compared to the controls. In order to test in a more systematic manner the hypothesis of a size-related response to cyanide, three different weight classes of trout of about 5, 16 and 27g were exposed to  $0.010\text{mg}\cdot\text{l}^{-1}$  HCN, fed a ration of  $1.0\%\cdot\text{day}^{-1}$  and held in a current of  $12.1\text{cm}\cdot\text{sec}^{-1}$ , the same as that used in Experiments 1, 2 and 3.

The results of Experiment 5 based on changes in wet and dry weights as well as in fat content are presented in Table 5. The wet weight average relative growth rates indicate that the 5g cyanide-poisoned fish grew faster than the controls whereas both the 16 and 27g controls grew better than their respective toxified groups. Student's t - tests, carried out on the data for each size group, revealed that a statistically significant difference between the control and cyanide-exposed fish existed only for the largest group of trout.

The dry weight gains of both the control and cyanide-poisoned groups were slightly higher than those for wet weight; the latter being more affected than the former, indicating a decrease in water content associated with the

Table 5. Average Relative Growth Rates based on wet and dry weights and fat content of different sizes of rainbow trout exposed to various concentrations of cyanide while swimming against a current of 12.1 cm-sec<sup>-1</sup> and being fed at a rate of 1.0% day<sup>-1</sup> in continuously renewed water at 10°C.

Expt. No.	Cyanide Cncc. (mg.l <sup>-1</sup> )	Initial Mean Wet Weight (g)	Final Mean Wet Weight (g)	Average Relative Growth Rate (Wet Weight) (mg.g <sup>-1</sup> .day <sup>-1</sup> )	Average Relative Growth Rate (Dry, Weight) (mg.g <sup>-1</sup> .day <sup>-1</sup> )	Average Relative Growth Rate (Fat Content) (mg.g <sup>-1</sup> .day <sup>-1</sup> )	"t" Value for Comparison of Growth of Control vs Cyanide-Toxified Group (Wet Weight)
5	0.000	5.56	6.17	5.44	6.55	5.06	-
	0.010	5.78	6.54	6.40	8.10	10.84	-0.70
	0.000	16.92	19.50	6.83	6.61	-1.53	-
	0.010	16.00	18.73	5.55	4.63	-7.70	1.28
	0.000	26.86	31.48	7.73	7.65	-0.63	-
	0.010	27.60	31.37	6.07	5.19	-8.43	1.98*

\* p < 0.05

accelerated growth rate. The dry weight growth rates of the 16 and 27g controls were about the same as those based on wet weight indicating true growth whereas the corresponding toxified groups had lower growth rates based on dry weight than wet weight, reflecting an uptake of water.

The cyanide-exposed fish in the smallest size class increased their fat content twice as much as the respective control group. Although the 16g controls experienced a loss of fat, the cyanide-poisoned fish lost 5 times as much fat. Similarly, for the largest weight class tested, the controls lost a small amount of fat while the cyanide-toxified fish lost 13 times as much.

The hypothesis that larger trout would be more seriously affected by cyanide poisoning than smaller ones was supported by the data of Experiment 5. It is also interesting to note that these results agreed well with those obtained in the previous experiments carried out under similar conditions, ( $1.0\% \cdot \text{day}^{-1}$  of food, a current of  $12.1 \text{cm} \cdot \text{sec}^{-1}$  and  $0.010 \text{mg} \cdot \text{l}^{-1}$  HCN). It appeared that very small fish would be stimulated to grow by cyanide, the effect diminishing with increasing size until a size was reached where growth was actually inhibited, the inhibition becoming progressively more pronounced with increasing size.

The metabolic rate of fish is related to size (Winberg, 1956), and this relation is described by the following allometric formula (Bertalanffy, 1957):



$$Y = aX^b$$

Where: Y = metabolic rate per unit time

X = body size (weight)

a = Y - intercept

b = weight exponent and slope

This formula may be rewritten in the following way:

$$\log Y = \log a + b \log X$$

Hence, if metabolic rate is plotted against body weight on log - log axes, a straight line is formed, the slope of which is b. Since growth rate is one way to measure metabolic rate, it was decided to plot on log - log axes the absolute wet and dry weight gains of each individual fish against its initial wet weight to compare the linear relation of control and cyanide poisoned fish ranging in size from 5 to 27g and fed a ration of  $1.0\% \cdot \text{day}^{-1}$  while swimming against a current of  $12.1 \text{cm} \cdot \text{sec}^{-1}$  using the data obtained through Experiments 2, 3, 4 and 5. The data of Experiment 1 were not used for this analysis because the cyanide concentration was not comparable being  $0.013 \text{mg} \cdot \text{l}^{-1}$  HCN instead of  $0.01 \text{mg} \cdot \text{l}^{-1}$  HCN in the following experiments.

In order to test the validity of the logarithmic transformation of the data and its linear regression, Bartlett's test for the homogeneity of variance was carried out on both raw and log transformed data (Brett and Glass, 1973). This test was used to compare the variances of both wet and dry weight gains of five weight classes within each

of the control and cyanide-exposed groups of fish and is presented in Table 6. For the untransformed data of both the wet and dry weight gains, the variance is not homogeneous and appears to be a progressively increasing function of body weight. For the log transformed data, the variance among weight groups is homogeneous for the wet weight gain of the cyanide-poisoned fish only. Homogeneity of variance is not indicated for the other log transformed data because the  $\chi^2$  is too large. However, for these other groups, the data can be considered to have homogeneous variance on the basis that the  $S^2$  values appear to be randomly distributed and because the computed values of  $\chi^2$  are not much greater than that required at the 5% level of significance. Hence, the log transformation of these data and subsequent least squares regression is deemed appropriate.

There are two pairs of regression lines illustrated in Figure 6; one pair is based on wet weight gains, the other on dry weight gains. Both pairs show the growth response of the control and cyanide-toxified trout over a wet weight range of from 5 to 27g. The slope of each line denotes the rate of change of growth with size. For those lines derived from wet weight gains, the slope of the controls is 1.32 while that of the cyanide-exposed fish is 1.01. On the basis of dry weight gains, the controls have a slope of 1.12 and the toxified group a slope of 0.67. It is important to note

Table 6. Results of Bartlett's test for homogeneity of variance. Data are given for control and cyanide-exposed rainbow trout, by weight class, and for raw and logarithmically transformed growth data expressed as wet and dry weight gain. The number of observations (n) is equal to the number of fish tested. The asterisk (\*) indicates homogeneous variance at the 5% level of significance for the appropriate df.

	Weight class (g)	Mean gain (g)	S <sup>2</sup>	Log mean gain	S <sup>2</sup>	n
Control Wet weight gain	5.56	0.6534	0.4024	0.1204	0.0475	29
	8.01	0.5105	0.3642	0.2848	0.1437	60
	11.59	1.3831	0.7759	0.0677	0.1369	29
	16.92	2.5770	2.0327	0.3472	0.0821	30
	26.86	4.6161	4.5414	0.6039	0.0666	28
		df=4 x <sup>2</sup> =84.79		df=4 x <sup>2</sup> =14.22		
HCN Wet weight gain	5.78	0.7983	0.2848	0.1459	0.1101	29
	8.02	0.6634	0.4712	0.1338	0.0774	58
	10.96	1.3052	0.5667	0.0879	0.0547	29
	16.00	2.0221	2.8439	0.2107	0.1485	29
	27.60	3.6628	4.0639	0.5014	0.0766	29
		df=4 x <sup>2</sup> =86.47		df=4 x <sup>2</sup> =8.59*		
Control Dry weight gain	5.56	0.1586	0.0175	0.7233	0.1523	29
	8.01	0.1283	0.0198	0.7926	0.2122	60
	11.59	0.3441	0.0381	0.5212	0.1275	29
	16.92	0.5500	0.0978	0.3086	0.0953	30
	26.86	0.9975	0.2249	0.0675	0.0751	28
		df=4 x <sup>2</sup> =83.20		df=4 x <sup>2</sup> = 11.95		
HCN Dry weight gain	5.78	0.2103	0.0131	0.6443	0.0768	29
	8.02	0.2043	0.0275	0.7227	0.1562	58
	10.96	0.3255	0.0273	0.5156	0.0830	29
	16.00	0.3741	0.1316	0.4778	0.2623	29
	27.60	0.6928	0.1933	0.2310	0.1290	29
		df=4 x <sup>2</sup> = 78.92		df=4 x <sup>2</sup> =14.49		

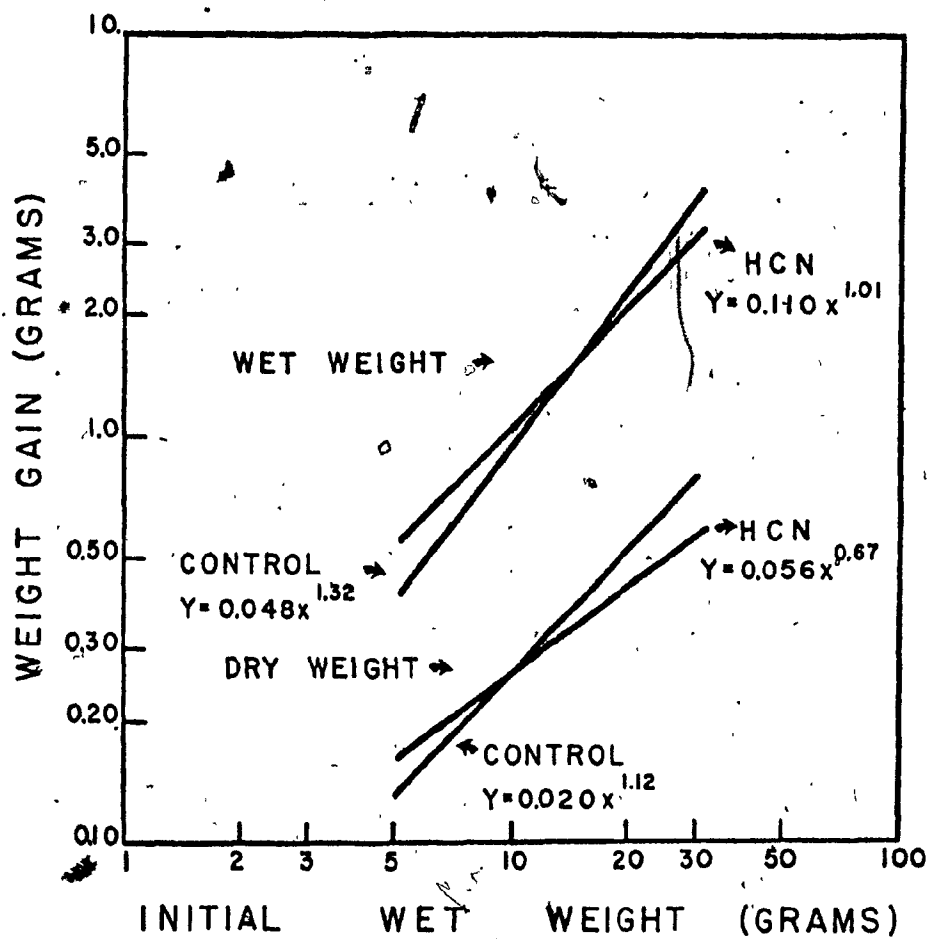


Figure 6. Regression lines relating wet and dry weight gains of control and cyanide-exposed juvenile rainbow trout to their initial wet weights ranging from 5 to 30g while swimming against a current of  $12.1\text{cm}\cdot\text{sec}^{-1}$  at  $10^\circ\text{C}$ .

that for both wet and dry weight gains the lines representing the controls and the cyanide-poisoned fish cross each other. What this means is that the growth of the small fish is stimulated by the cyanide over that of the control group, whereas the growth of the large fish is depressed. For fish of 10 - 15g there is no difference. It should be pointed out that the wet weight lines cross at an initial wet weight of about 15g whereas the dry weight lines cross at about 10g. This difference is due largely to a decrease in slope of the line representing the toxified fish from 1.01 to 0.67 which was caused by the larger fish increasing their water content while the smaller fish decreased their water content thereby masking the true rate of growth.

The parameter values of the regression lines illustrated in Figure 6 are presented in Table 7. The slopes of the lines representing the control and cyanide-poisoned fish were statistically compared on both a wet and dry weight gain basis and in both cases, there was a significant difference. The correlation coefficients measuring the strength of the association between weight gain and initial wet weight were highly significant based on both wet and dry weight gains of the control and toxified groups. However, the relatively low values of the correlation coefficients themselves reflect a considerable amount of variation in the growth response. The sources of variation are several. Differences in age, sex and season, not taken into account in this analysis, may

Table 7. Parameter values of regression lines based on the log-log transformation of the data of wet and dry weight gains

	Equation	Regression Coefficient	95% Confidence Limits For Regression Coefficient	Correlation Coefficient	Test Of Difference Between Slopes
Control (Wet Wt.)	$\log Y = -1.32 + 1.32 \log X$	1.32**	1.11 < 1.32 < 1.52	0.69**	3.16*
HCN (Wet Wt.)	$\log Y = -0.98 + 1.01 \log X$	1.01**	0.83 < 1.01 < 1.19	0.64**	
Control (Dry Wt.)	$\log Y = -1.70 + 1.12 \log X$	1.12**	0.89 < 1.12 < 1.36	0.58**	3.73*
HCN (Dry Wt.)	$\log Y = -1.25 + 0.67 \log X$	0.67**	0.44 < 0.67 < 0.91	0.39**	

\* p < 0.05  
 \*\* p < 0.00001

have contributed as well as genetic differences both within and among groups of fish.

#### Effect of initial size on fat gain

Figure 7 shows gains or losses of fat in the control and cyanide-exposed fish used in the regression analysis. A semi-log plot of average relative growth rate vs initial wet weight was used instead of a log - log graph because of the presence of negative values of fat changes.

The curve for control fish shows that the small individuals increasingly gained fat up to a size of about 11g whereas the bigger ones lost fat just maintaining themselves near a zero rate of growth. The curve for cyanide-poisoned trout reveals that the small fish had a faster buildup of fat content up to the same level as that for the 11g control fish, but then a drastic loss in fat was experienced by fish larger than 11g such that they lost substantially more than the controls. The curves cross over at an initial wet weight of 11g where there is no difference in fat gain between the control and toxified groups. It should be noted that the changes in fat content follow those based on wet and dry weight from the regression analysis very closely (see Figure 6).

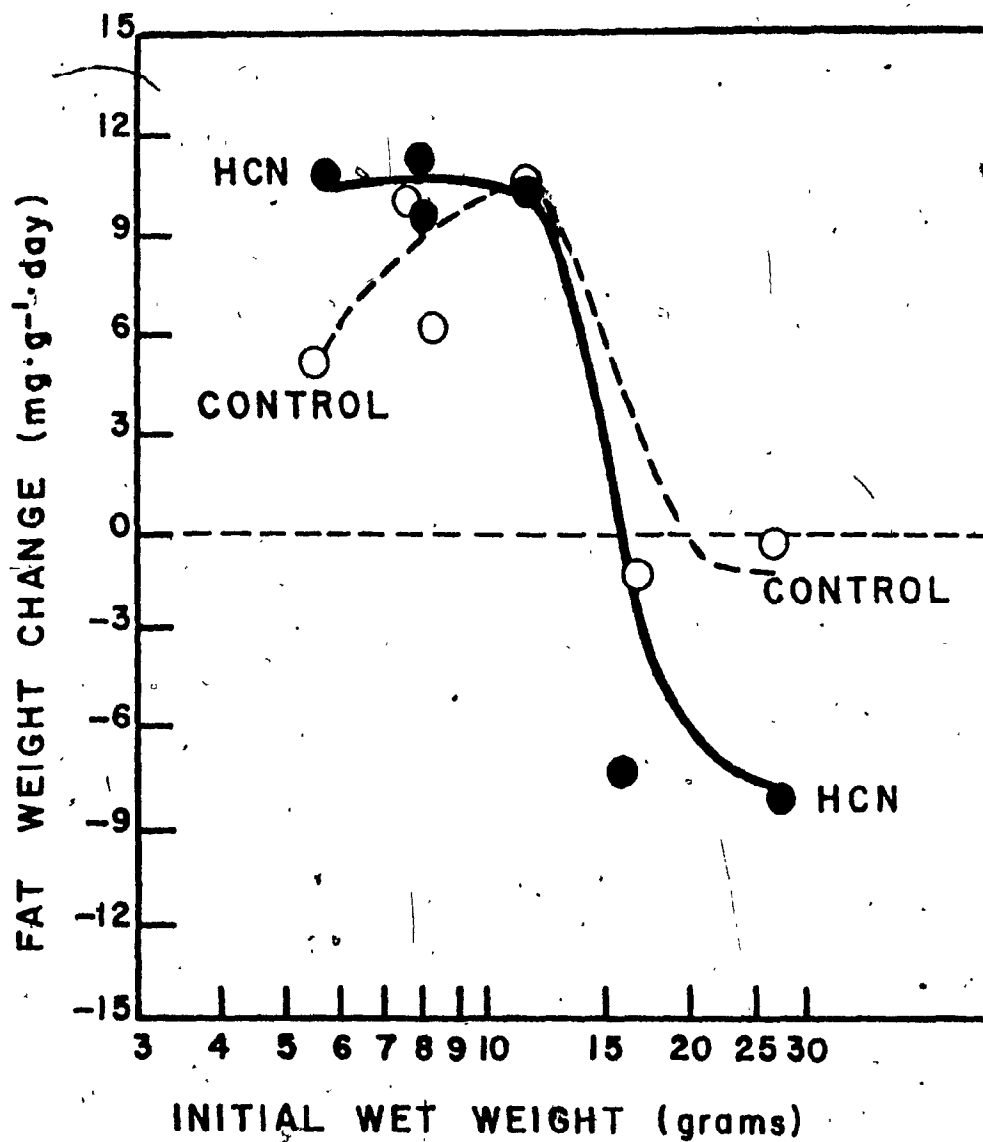


Figure 7. The relationship between fat weight changes of control and cyanide-exposed rainbow trout and their initial wet weights ranging from 5 to 30g while swimming against a current of  $12.1\text{cm}\cdot\text{sec}^{-1}$  at  $10^{\circ}\text{C}$ .



## DISCUSSION

## Physiological Implications

Overall, the results of this study suggest that modifying factors of metabolic rate such as size and activity alter the response of fish to chronic cyanide poisoning, acting directly on energy supply systems.

The absolute body size of an animal not only governs its metabolic rate but many physiological and morphological parameters as well (Adolf, 1949; Schmidt-Nielsen, 1975. p.255). The equation describing this dependence on body size is known as the allometric formula (Bertalanffy, 1957):

$$\log Y = \log a + b \log X$$

where Y is the size-dependent variable, X is the body size and a and b are constants. Growth can be described by this formula and if one plots weight gain against body weight on log - log axes, a straight line is obtained, where the value of the slope describes the rate of change of growth with size. For example, if  $b = 0.67$ , then the growth of the animal is proportional to  $2/3$  the power of weight or to its surface area. If  $b = 1$ , then growth is directly proportional to weight. For values in between, growth is intermediate between proportionality to surface area and proportionality to weight, revealing a disproportionate increase in growth with increasing size.

The metabolic rate of fish is commonly measured

by monitoring oxygen consumption but this measurement can be achieved through growth studies (Elliott, 1976). This was originally proposed by Paloheimo and Dickie (1966) who concluded, after an analysis of the literature, that the values of the weight exponent in oxygen consumption experiments were very close to those obtained through feeding experiments. Given the interest of this approach, it was adopted in the present study of allometric factors modifying chronic cyanide toxicity in juvenile rainbow trout.

The slopes of the wet weight regression lines (Figure 6) describing the growth of the control and cyanide-exposed trout were determined to be 1.32 and 1.01 respectively. However, the cyanide-poisoned fish experienced a disruption in their water content, thereby masking the true rate of growth. Speyer (1975) and Dixon (1975) noted the same effect in earlier growth studies using rainbow trout subjected to 0.01, 0.02 and 0.03mg·l<sup>-1</sup> HCN. Furthermore, Leduc and Chan (1975) have shown that rainbow trout exposed to concentrations of cyanide as low as 0.01mg·l<sup>-1</sup> HCN suffered an impaired ability to osmoregulate. Hence, this apparent cyanide-induced disruption of osmoregulation and consequent water imbalance can lead to a misinterpretation of the growth data. This problem was overcome by examining the slopes of the dry weight regression lines. The slope for the control fish was 1.12, a value very close to the one of

T.O. previously reported by Rao (1968) and Brett and Glass (1973), describing the active metabolic rate of rainbow trout and sockeye salmon, Oncorhynchus nerka, respectively. These results suggest that the growth of the control fish and hence, metabolic rate, were proportional to body weight. On the other hand, the slope representing the cyanide-poisoned fish with a value of 0.67 appears to indicate a growth proportional to body surface.

The slopes of the growth-size regression lines (Figure 6) differentiate the ability to grow between large and small fish in the presence of cyanide. Growth directly proportional to weight is faster than growth proportional to surface area and favours the large individuals. Consequently, the large fish were more severely affected by cyanide than the smaller ones, since cyanide induced growth proportional to body surface. However, growth proportional to surface area favours small fish such that the cyanide-exposed ones grew better than the controls. At an intermediate size of about 11g, there was no difference in growth between the control and cyanide-poisoned trout. Thus, there exists a change in tolerance to cyanide with increasing size.

#### Response to varying ratios

The size-related response to cyanide was first suspected from the results of Experiments 1, 2 and 3 where

cyanide markedly increased the food maintenance requirements of 18g trout but not of the 8g ones.

To obtain an accurate measurement of the food maintenance requirement, the activity of the fish must be regulated for Leduc (1966) observed that cyanide-poisoned coho salmon, Oncorhynchus kisutch, had lowered their activity, thereby contributing to an increased food conversion rate and thus helping to overcome the cyanide poisoning. In this study, uniform forced swimming at  $12.1 \text{ cm} \cdot \text{sec}^{-1}$  eliminated that possibility.

On a dry weight basis, cyanide increased the food maintenance requirement of the 18g trout by 2 - 3 times. The effect of cyanide on 8g fish was visible only at the zero feeding level (starvation) where increased weight losses relative to the controls occurred, although the difference was only statistically significant in Experiment 3. This observation, comparable to that of Leduc (1966) on cyanide-poisoned starving cichlids, suggests that cyanide interferes with the transformation of body energy reserves for maintenance, but not with that of food-derived energy. Thus the cost of basal (starvation) metabolism was slightly increased by cyanide, most likely due to impaired biological oxidation of body reserves (lipids and proteins) whereas when food is used for maintenance, glycolysis can insure an immediate energy source. Kovacs (1978) has indeed measured a stimulation

of glycolysis in rainbow trout fingerlings subjected to  $.015\text{mg}\cdot\text{l}^{-1}$  HCN at  $6^{\circ}\text{C}$ .

Cyanide had no effect on the food maintenance requirement of the 8g fish based on dry weight. However, it should be remembered that because of a procedural error, the cyanide concentration in the first experiment (18g fish) was  $0.013\text{mg}\cdot\text{l}^{-1}$  HCN compared to  $0.010\text{mg}\cdot\text{l}^{-1}$  HCN in Experiments 2 and 3 (8g fish). It is unlikely though, that the higher cyanide concentration could explain such a marked difference between the two size groups, all other conditions being equal. For example, in a similar experimental regime except for the lack of current, Dixon (1975) and Kovacs (1978) using 12 and 20g rainbow trout respectively fed rations of  $2.5\%\cdot\text{day}^{-1}$ , did not observe more than a 10% reduction in dry weight growth rate from  $0.010$  to  $0.013\text{mg}\cdot\text{l}^{-1}$  HCN. Yet in this study, the difference in dry weight growth rate between the 8 and 18g cyanide-exposed fish at the  $1.0\%\cdot\text{day}^{-1}$  feeding level was 159%, suggesting a size-related phenomenon. It would appear then that cyanide was acting as a stressing factor on the 18g fish, increasing the amount of energy required for maintenance and hence, reducing the amount available for growth.

A higher food maintenance requirement in smaller fish (Figure 4) has also been observed by Wurtsbaugh and Davis (1977b) who found that the maintenance ration of

rainbow trout, expressed as % dry weight of fish·day<sup>-1</sup>, swimming at 1.0 - 1.2L·sec<sup>-1</sup> increased from 3.7 to 5.3%·day<sup>-1</sup> with a decrease in size from 3.4 to 0.6g. This is due in part, to the higher weight specific metabolic rate of the smaller fish, leaving less energy available for growth. In addition, since both size groups were held at the same current velocity, the swimming speed in lengths per second was 1.4 for the 8g trout as opposed to 1.0 for the 18g trout, thus imposing a higher cost of swimming on the small fish.

The differential effect of cyanide on the growth and food maintenance requirements between the small and large trout seemed to have been related to an elevated weight specific metabolic rate and an increased relative activity of the small fish contributing to a higher oxygenation of the tissues and/or elimination of cyanide.

#### Response to exercise

The results of Experiment 4 using uniform size fish of 11g at three different current speeds did not contribute much in answering these questions, but did not contradict the previous results either. The 11g fish subjected to cyanide grew at about the same rate as the controls at swimming speeds of 6.7 and 12.1 cm·sec<sup>-1</sup> but grew significantly better than the control group at 20.4cm·sec<sup>-1</sup>.

Finally, the results of Experiment 5 further emphasized the effect of size where small cyanide-exposed trout (5g) grew slightly better than the controls, whereas the large poisoned trout (16 & 27g) suffered reductions in growth relative to the control groups.

There have been a number of cases where cyanide has promoted faster weight gains. Negilski (1973) subjected juvenile chinook salmon, Oncorhynchus tshawytscha, weighing about 1.5g to  $0.01\text{mg}\cdot\text{l}^{-1}$  HCN for several weeks in an artificial stream environment with a current speed of  $24\text{cm}\cdot\text{sec}^{-1}$  and found that in two separate experiments the cyanide-exposed salmon grew better than the controls. This phenomenon of growth stimulation by cyanide was also reported in growing cichlids exposed at  $25^{\circ}\text{C}$  and in developing Atlantic salmon sac fry, Salmo salar, subjected to low concentrations of the poison (Leduc, 1966, 1978). In the latter case the faster growth of the cyanide-exposed sac fry was related to their smaller size at hatching which in turn was due to previous exposure to cyanide during incubation.

An increase in temperature, which also activates metabolism in fish; has a determining influence on cyanide toxicity at lethal and sublethal levels. Kovacs (1978) has confirmed that the toxicity of slowly lethal concentrations of cyanide to rainbow trout is inversely related to temperature and metabolic rate. In the range of  $6$  to  $18^{\circ}\text{C}$  the

food maintenance requirements increased by a factor of 2.1 and the 96hr. LC50 by 2.4. He also demonstrated the same phenomenon at sublethal levels by measuring the effects on growth and swimming ability. It therefore becomes apparent that a higher total metabolic rate brought about through forced swimming or a rise in temperature is a modifying factor of cyanide toxicity.

#### Response of lipid metabolism

Given the high energetic implication of fat metabolism, variation of lipids could give some explanation to the general observations on growth previously discussed. The changes in fat content (See Figure 7) closely parallel the overall changes in wet and dry weight (See Figure 6). But why would the gain in fat of the controls reach a maximum in 11g fish and then decline in larger ones? It should be remembered that Figure 7 shows the changes in lipid, not the total fat content of these fish. If the curve of the control trout kept on rising, eventually a point would be reached where the dry weight of the fish would be composed of 100% fat, which is of course, impossible.

The observed fat weight changes should reflect either changes in metabolic pathways or changes in relative metabolic costs to small and large fish. For example, it is known that under prolonged exercise at high swimming speeds that proteins are used as an energy source whereas



at lower velocities, fat and protein are used (Krueger et al., 1968).

With regards to the effects of cyanide on growing fish Dixon (1975) and Speyer (1975) both found decreased fat levels associated with depressed growth rates of cyanide-poisoned rainbow trout, ranging in size from 4 to 25g, held in flow-through tanks (no current), which suggested that cyanide invariably disrupted normal fat biosynthesis through partial inhibition of oxidative metabolism. This was quite different from what was observed here where cyanide produced more fat than the controls in small trout (5g), less in large ones (27g) and the same amount in intermediate 11g trout (See Figure 7).

These results would tend to imply that when a high active metabolic rate is forced on fish, cyanide, by reducing the much needed aerobic metabolism, would activate glycolysis as an alternate source of energy. Kovacs (1978) has shown that glycolysis in rainbow trout is activated by cyanide. It is also known that glycolysis in fish shunts a greater portion of substrates away from the Krebs cycle into the acetyl - CoA pool used for lipid synthesis (Prosser, 1973. p.226). Hence, the smaller fish with a higher total metabolic rate would be able to initiate glycolysis more quickly than larger ones, thereby permitting a buildup of lipid reserves. In large fish however, with a lower total metabolic rate and being close to a fat maintenance level,

cyanide would prevent any new fat deposition.

#### Allometric response

The allometric response of fish to respiratory deficiency has long been established. Wells (1913) subjected different species and sizes of fish to tanks containing sufficiently low levels of oxygen to cause death and measured their respective survival times. He found that for the common shiner, Notropis cornutus, ranging in size from 0.6 to 21.0g that the average dying time per gram declined from 25.0 to 2.1 minutes. Similarly for rock bass, Ambloplites rupestris, from 1.9 to 31.0g in weight, the average dying time per gram decreased from 10.5 to 1.4 minutes, indicating that small fish were more resistant, per unit weight, to low concentrations of oxygen. Furthermore, Herbert and Merckens (1952), observed that the survival time of juvenile rainbow trout immersed in a lethal solution of cyanide decreased from 39.0 to 16.0 minutes with an increase in length from 5.75 to 17.25cm. Hence, lethal studies concerning low levels of oxygen and the inhibition of utilization of oxygen by cyanide both show that smaller fish are more tolerant than larger ones.

In this study, using a sublethal concentration of cyanide and creating an unfavourable condition for growth, the growth of the cyanide-poisoned trout on a dry weight basis was proportional to surface area, indicating that

smaller trout are more resistant than larger ones. It is also interesting to note that the results of Zeisberger (1961), using the common carp, Cyprinus carpio, suggest that under unfavourable conditions for growth during winter that the slope of the line relating metabolic rate to size (1 - 100g) was approximately equal to 0.67 whereas in the summer under favourable growth conditions the slope was about 1.0.

Hughes (1970) has established for several species of fish on a log - log plot that the slope of the line relating gill area to body weight is equal to 0.8, showing that smaller fish have a larger gill area per unit weight than larger ones. Hence, they would be able to consume more oxygen on a weight specific basis than larger fish. Beamish (1964) has in fact determined for several species that the oxygen consumption per unit weight is greater for smaller than larger fish. For example, the oxygen consumption at 10°C of a brown trout weighing 60g is approximately  $0.083\text{mg}\cdot\text{g}^{-1}\cdot\text{hr}^{-1}$  as compared to  $0.067\text{mg}\cdot\text{g}^{-1}\cdot\text{hr}^{-1}$  for a 300g fish. In addition, Jones (1971), has calculated that with an increase in size of the fish, there occurs an unproportional increase in cost of gill ventilation. Thus, the larger cyanide-exposed trout having a greater maintenance energy cost would have less energy available for growth. The greater oxygen consumption per unit weight and lower cost of gill ventilation of the smaller fish may help to

explain how they were able to cope with cyanide poisoning. On the other hand though, the faster metabolic rate of the smaller fish coupled with their larger gill area on a weight specific basis would also allow greater intake of cyanide per unit weight than in larger fish. Yet in this study, the smaller cyanide-exposed fish grew better than the controls. Two important concepts of toxicology should be considered here: uptake and clearance rates. In the small fish a higher uptake rate would have a double effect:

1. to partly inhibit oxidative metabolism, an effect compensated by a high respiratory/body size capacity, and
2. to activate glycolysis and stimulate fat deposition from the food as previously discussed. The net effect would be a lower Specific Dynamic Action (SDA) (Warren, 1972. p. 142) and higher caloric content.

In the large fish the lower cyanide uptake would be however, accompanied with a lower respiratory/body size capacity thus reducing biological oxidation and causing a higher SDA particularly depleting fat reserves.

With regards to clearance rate, it is not unreasonable to speculate that the higher metabolism of the small fish could lead to a faster detoxication and/or elimination of cyanide. In mammals, cyanide can be detoxified by the action of rhodanese, an enzyme found abundantly in the liver, which catalyses the reaction between thiosulfate and cyanide, forming the nontoxic thiocyanate which is eliminated

slowly and irregularly in the urine (West et al., 1966. p. 545). There is some evidence that the rhodanese mediated system is indeed present in fish for Achard and Binet (1934) found that carp, Cyprinus carpio Linnaeus, in the presence of thiosulphate experienced an increased survival time in lethal solutions of cyanide.

No definite explanation can be given but in the size range of 5 to 30g there appeared to be a transition where the positive and negative effects of cyanide on growth balanced each other in 11g fish. We cannot say why but it is interesting to note that in Figure 3 of Brett and Glass' (1973) paper that at 10.5°C the active metabolic rate isopleth of 700mg O<sub>2</sub>·hr<sup>-1</sup> deviates from the horizontal at a size of about 15g. This means that beyond that size, sockeye salmon are not able to maintain a maximum activity of 700mg O<sub>2</sub>·hr<sup>-1</sup> at 10.5°C due to limiting respiratory/circulation capacity. Given the similarity of the two species tested at the same temperature the "no effect" size of 11g found with cyanide-exposed rainbow trout may not be coincidental but reflect the true allometric response to a respiratory poison.

Ecological Significance

From the 20-day growth experiments carried out in this study, it was apparent that the toxicity of cyanide

was dependent on the size of the fish; large fish being more sensitive than small ones. Although this was a laboratory project with rigorously controlled conditions, some attempt was made to incorporate conditions from the natural environment. The use of a current that the fish had to swim against as well as a series of restricted rations approximated the natural aquatic system in the sense that fish have to swim to maintain themselves in the current to escape predators and catch food, the supply of which is usually limited. The response of the larger trout revealed that regardless of ration size, they were unable to cope with the cyanide and hence, in nature, would be seriously adversely affected by the presence of cyanide.

At the starvation ration level, the small cyanide-exposed trout lost more weight than the controls. Since most fish experience a severe seasonal depletion of body constituents (Love, 1970. p. 222), and/or a mobilization of fat, (Newsome & Leduc, 1975), cyanide, by acting as a stressor, could cause the fish to starve to death or alternatively become so weak that they would be increasingly susceptible to disease, parasitism and predation.

The small poisoned trout receiving rations of 1.0 and 2.0%·day<sup>-1</sup> grew as well or better than the controls over the 20-day experimental period. Similarly, Dixon (1975) observed that at a ration level of 2.5%·day<sup>-1</sup> 12g rainbow trout subjected to 0.01mg·l<sup>-1</sup> HCN grew as well as

the controls. He also noted however that this same group of fish suffered a certain amount of necrobiosis of their liver hepatocytes, revealing that although the measurement of growth is a good overall indicator of cyanide toxicity, it is not the most sensitive. Other sublethal tests of the toxicity of cyanide include swimming stamina tests and tests on the reproductive capability of fish. Both Neil (1957) and Broderius (1970) found that brook trout and coho salmon respectively required 14 to 20 days to recover their swimming capacity following exposure to  $0.01\text{mg}\cdot\text{l}^{-1}$  HCN, indicating serious physiological impairment and reduction of the scope for activity (Fry, 1947).

One of the effects of cyanide on the reproductive capability of fish is the prevention of maturation of testicular germ cells in rainbow trout subjected to a concentration of  $0.01\text{mg}\cdot\text{l}^{-1}$  HCN, as was demonstrated by Ruby and Dixon (1974). Also, the deposition of yolk, which consists largely of fat, in rainbow trout ovaries has been shown to be impaired by concentrations as low as  $0.01\text{mg}\cdot\text{l}^{-1}$  HCN (Lesniak, 1977). Thus, the reduction in fat content of the large toxified trout observed in this study after a relatively short period of exposure coupled with a decreased ability to reproduce, poses a serious threat to the survival of the entire population.

This study has something unique compared to many

other studies summarized by Doudoroff (1976) and Leduc (1977) in that, except for Negilski (1973), all were performed in semi-static conditions i.e. a flow-through system with no current. It was on these experiments that the National Academy of Science and National Academy of Engineering (1974, p. 190) based their recommendation that cyanide concentrations should not exceed  $0.005\text{mg}\cdot\text{l}^{-1}$  HCN at any time or place. Considering that most often toxic effluents are discharged in streams, to be realistic, ecotoxicological research should have this variable tested:

Although limited in scope, the results of the present study have shown that exercise could be a serious modifying factor of the toxicity of cyanide to juvenile rainbow trout.

There is a dearth of information of the influence of continuous moderate exercise on the growth of fish under normal and toxic conditions. The design and execution of long term experiments in swimming chambers are certainly more elaborate than under semi-static test conditions but essential to the definition of ecologically sound water quality objectives with regards not only to cyanide but to all toxicants affecting stream dwelling fishes.



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