Acute and Sublethal Toxicity of Cyanide to Exercised and Non-Exercised Rainbow Trout (*Salmo gairdneri*) at Different Times of the Year.

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A Thesis

in

The Department

of

Biological Sciences

Presented in Partial Fulfilment of the Requirements
for the degree of Master of Science at
Concordia University
Montréal, Québec, Canada

October 1984

ABSTRACT

Acute and Sublethal Toxicity of Cyanide to Exercised and Non-Exercised Rainbow Trout (Salmo gairdneri) at Different Times of the Year.

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The lethal toxicity (96-h LC50) of cyanide (HCN) to fingerling rainbow trout (Salmo gairdneri) varied seasonally and with exercise (swimming at 1 body length/sec). The trout were acclimated to 12°C test temperature for 3-4 weeks and kept under a 12 hour photoperiod. During summer experiments, there was no significant difference in LC50s between exercised and non-exercised trout (0.058 and 0.055 mg/L HCN respectively). However, in winter trials, the LC50 for exercised trout remained fairly constant at 0.052 while the non-exercised trout value dropped significantly to 0.043 mg/L HCN. Median survival times were similar in the summer while those of the exercised fish exceeded that of the non-exercised fish by up to 100% during the winter assay. Trout tested at 18°C also maintained a fairly constant response (96-h LC50) from summer to winter as did the exercised trout. Increase of the acclimation period of the non-exercised trout from 4 weeks to 10 weeks during the winter appears to induce a "summer" like response.

A 20-day growth experiment at sublethal concentrations

of cyanide was carried out. In the absense of cyanide non-exercised trout grew faster (+28%) than the exercised trout. However, this growth deficit among the exercised trout is overcome when cyanide concentrations of 0.005, 0.010, and 0.020 mg/L HCN are introduced into the water as they show a growth stimulation of 32%. Growth is depressed only at the highest cyanide concentration of 0.020 mg/L HCN tested on the non-exercised trout.

Liver glycogen and blood plasma thiocyanate levels were also measured in exercised and non-exercised trout during 15 days of exposure to sublethal concentrations of cyanide.

ACKNOWLEDGEMENTS

This research study was supported by the Department of Education, Province of Quebec (FCAC). Support was also provided from a Concordia Fellowship and an NSERC Scholarship.

I would like to extend my sincere thanks and appreciation to my advisor Dr. Gérard Leduc for valuable assistance and criticism in the preparation and writing of this thesis. I would also like to extend my thanks to Dr. Perry Anderson and Dr. Daphne Fairbairn for their much needed advice and guidance.

Special thanks go to Pierre Raymond for his personnal help and encouragement. Throughout this study, fellow graduate students Dan Cyr, Jim Nagler, Martha Carillo, Masaaki Sawada and Gonum Reddy, provided insight and support.

I would also like to thank my parents, Duncan and Florance McGeachy, and my family for their continued support and encouragement throughout my entire academic studies.

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INTRODUCTION

Activity and seasonal changes, as modifying factors of toxicity, have been given relatively little attention in establishing water quality criteria for toxic pollutants. The purpose of this study was to compare and evaluate the response of exercised and non-exercised rainbow trout (Salmo gairdneri Richardson) exposed to lethal and sublethal levels of cyanide at different seasons of the year.

Swimming performance is used as an important criterion in the evaluation of toxic responses of fish to various pollutants (Brett 1967, Sprague 1971). Cyanide (Neil 1957, Broderius 1970, Kovacs and Leduc 1982b), pulpwood fiber (MacLeod and Smith 1966), sodium pentachlorophenate (Oseid and Smith 1972) and fenitrothion (Peterson 1974) have been shown to detrimentally affect swimming performance during and/or following exposure to the toxicants. Negilski (1973) and McCracken and Leduc (1980) have studied the growth response of exercised young salmonids to chronic cyanide poisoning but there is no information on the effect of swimming on the acute toxicity of cyanide.

It appears that the potency of cyanide at lethal and sublethal levels is inversely related to metabolic rate. Previous work on cyanide by Kovacs and Leduc (1982a) has shown that rainbow trout tolerance is temperature dependent, with sensitivity increasing at lower temperatures. Rao (1968) observed a 20% reduction in total metabolism when

rainbow trout were transferred from freshwater to a salinity of 17‰. A euryhaline fish exposed to cyanide in saltwater should be more susceptible due to reduced metabolism and this was observed by Broderius (1973) when he compared the toxicity of cyanide to the threespined stickleback (Gasterosteus sp.) in salt and freshwater. Survival times in saltwater were much shorter than in freshwater.

The influence of cyanide on growth has also been related to metabolic rates of the fish being tested. McCracken and Leduc (1980) observed that smaller trout (higher specific metabolic rate) grew faster than control fish of the same weight and faster than larger ones (lower specific metabolic rate) when exposed to cyanide in swimming chambers. Negilski (1973) using artificial streams observed faster growth of juvenile chinook salmon (Oncorhynchus tshawytscha) at 0.010 mg/L HCN. Kovacs and Leduc (1982b) also found growth to be more reduced by cyanide at 6°C than at 18°C.

Accordingly, changes in metabolic rate induced by exercise should change the sensitivity of fish to cyanide and it was decided to test this hypothesis at the lethal and sublethal levels. The first objective of this study was to compare the 96-h LC50 of exercised and non-exercised rainbow trout and to measure the effects of exercise on the growth of cyanide exposed rainbow trout at 12°C. In the hope of better understanding the responses of cyanide toxicity on energetics it was decided to measure the liver glycogen

level which is a good indicator of energy reserves since Kovacs and Leduc (1982b) observed greater glycogen utilization during cyanide exposure at 6°C than at 12 and 18°C.

The second objective of this study was to verify the effect of season on cyanide toxicity. Fish of temperate regions are exposed to seasonal changes which may influence their biochemistry and thus, may affect their toxic response. Many researchers have observed cycles in the physiological and behavioral patterns of fish that have been attributed to the seasons. Hoar (1959) notes that resistance to sudden chilling in goldfish (Carassius auratus) increases during the winter while resistance to heat is elevated in the summer. Hoar (1959) suggests that yearly physiological cycles play a vital role in temperate fish. Metabolic rates in brook trout (Salvelinus fontinalis) have been shown to decrease during the winter even though the same temperatures are maintained throughout the year (Beamish 1964b). As well, Withey and Saunders (1973) observed lower standard rates of oxygen consumption in Atlantic salmon (Salmo salar) subjected to a reciprocal photoperiod regime (decreasing day length from early March and increasing day length from late June) than with those maintained under simulated natural conditions. This work suggests some sort of photoperiod and cyclical involvement in fish respiration. Thyroid hormones are known to increase electron transport activity as well as respiration (Tepperman 1980, Massey and Smith 1968) which

could influence cyanide toxicity due to cyanide's effect on the respiratory chain. Work by White and Henderson (1977) has shown thyroid hormones in brook trout to be lowest during the winter months. Smith et al (1978) have also shown cyanide toxicity to be influenced by temperature as well as season and their results suggest that cyanide is more harmful during the winter period. The Ministry of Technology (in Brown, 1968) reported seasonal variations by a factor of 2.5 in 48-h LC50 values for rainbow trout exposed to ammonia, phenol and zinc. Unfortunately this study did not indicate in which season the toxicants were most toxic.

This study investigated the influence of season by testing lethal responses in trout exposed to cyanide at various times of the year. It was hypothesised that trout tested during the summer season would be more resistant to cyanide than trout tested during the winter season, due to a general lowering of metabolic rate during the winter months.

Measurements of thiocyanate were undertaken in the hope of better understanding the role of cyanide toxicity in exercised and non-exercised trout. The conversion of cyanide to the less toxic thiocyanate is catalyzed by the enzyme rhodanese which is most active in the liver (Westley 1981). This enzyme has been isolated in fish liver, kidneys and gills (Schievelbein et al 1969). Most of the thiocyanate studies completed to date have dealt with mammals and birds (Bourdoux et al 1978, Davis 1981). These

studies have shown that soon after exposure to cyanide, levels of thiocyanate in the blood and urine increase as a result of cyanide conversion to thiocyanate. A more recent study by Raymond (1984) has demonstrated that rainbow trout, exposed to sublethal levels of cyanide for 20 days, showed marked increases in plasma thiocyanate concentrations within 48 hours of exposure. Hence, cyanide is successfully detoxified to thiocyanate which is an antithyroid agent that may play an important role in thyroid function (Tepperman 1980). It is important to keep in mind that thiocyanate accumulation depends upon the uptake and conversion rates of cyanide and its excretion from the fish, all of which may be different in exercised and non-exercised trout.

To test the hypothesis that exercised rainbow trout are more resistant and better able to withstand cyanide than non-exercised rainbow trout, bioassays at lethal and sublethal levels were conducted on exercised and non-exercised trout at different times of the year. Lethal testing looked at changes in the 96-h LC50 value. Sublethal responses were examined by monitoring growth, liver glycogen, and blood plasma thiocyanate levels over 15 to 20 day exposures at 12°C. In the course of experimentation, other minor hypotheses arose from the results and were tested as well. These hypotheses are presented later when appropriate.

MATERIALS, APPARATUS AND METHODS

Test Organisms

Fingerling rainbow trout (Salmo gairdneri Richardson) used for this study were purchased from La Pisciculture Mt. Sutton, Sutton, Quebec. Upon arrival at Concordia University, the fish were transferred to 200-L fibreglass, oval shaped, holding tanks at a density of 160 fish per tank. The holding tanks were supplied with a continuous flow of water of approximately 2 L/min at a temperature of 12±1°C. The water supply was dechlorinated City of Montreal water delivered through PVC piping and with the chemical characteristics shown in Table 1. The fish were fed daily ad libitum with Ewos Trout Chow and/or Purina Trout Chow size 3 and 4 pellets during this holding period. The photoperiod was maintained at 12 hours light and 12 hours dark. Mortality during holding was less than 1%.

Except when mentioned, all fish were held for 10 to 14 days under the above conditions in the 200-L holding tanks prior to testing. After this initial holding period the fish to be tested were randomly selected (using a random numbers table) and transferred to their appropriate bioassay tanks for acclimation to experimental conditions for a further two weeks.

Bioassay Apparatus

There were two types of bioassay tanks used in this

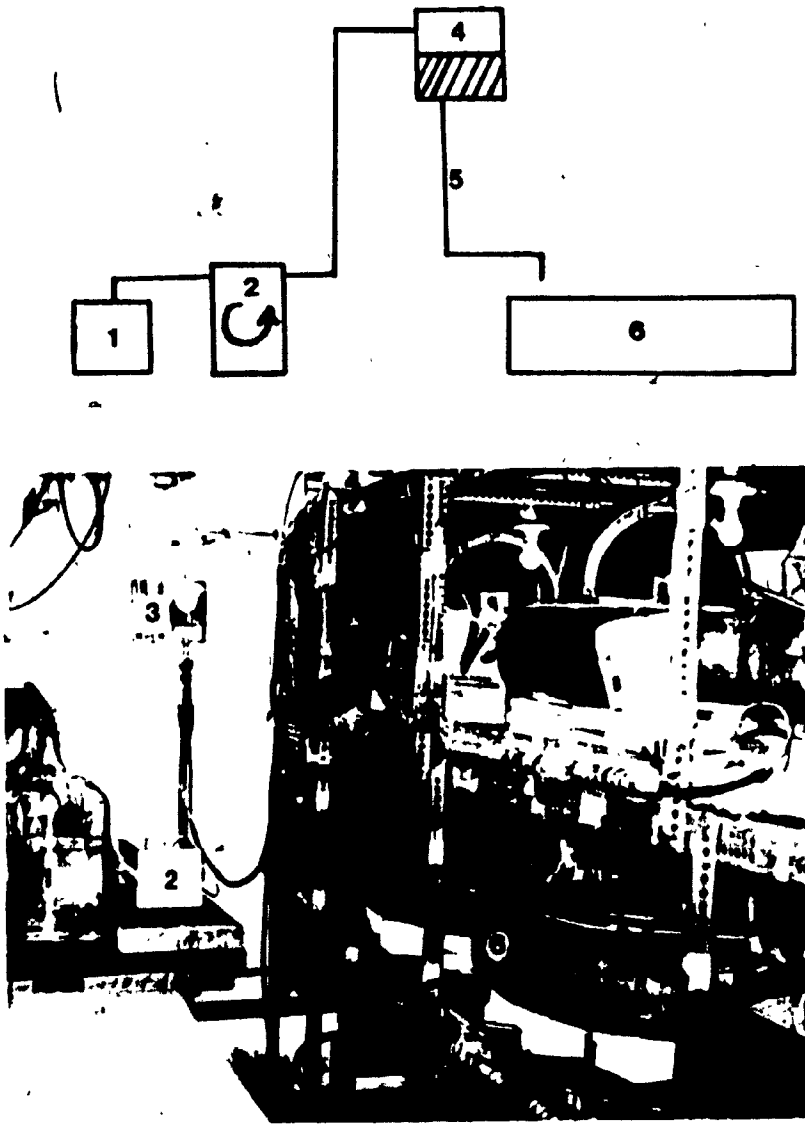


Figure 1.

Schematic diagram and photograph of the experimental tanks used to exercise the trout showing the annular tanks, cyanide stock bottles and cassette pump which metered the cyanide into the tanks.

1) Cyanide stock solution bottle
2) Cassette pump
3) Timer-switch (Photoperiod)
4) Small head tank with cyanide
5) Microtubes from (4) to annular tanks
6) Annular tank

Figure 2.

Photograph of the experimental tanks used for the non-exercised trout showing the rectangular tanks and Mariotte bottles which metered cyanide into the tanks.

1) Mariotte bottle for metering the cyanide
2) Bioassay tank with styrofoam insulation and black plastic
3) Cross-section of tank showing black plastic

study, one for the exercised trout and the other for the non-exercised. The exercised trout were in annular swimming chambers (Kruzynski 1972) equipped with electric motor-driven paddle wheels creating a current of about 12 cm/sec. (Figure 1). These 87-L fibreglass tanks have a diameter of about 102 cm, a depth of 21 cm and a maximum width of 23 cm. Each tank was connected to a constant flow-through system and the water temperature was maintained at 12±1° C. A Manostat Cassette Pump (Manostat Corp., New York, N.Y.) was used to deliver stock solutions of cyanide to a small head tank above the annular chambers. The volume of these head tanks were kept constant via an overflow outlet since the cyanide stock solutions were pumped into these small head tanks faster than the solution leaving these head tanks. Microtubes were used to deliver the cyanide from these small head tanks to the annular chambers. Thus with a constant level maintained in the small head tanks, pressure at the point of exit from the microtubes (just above the waters surface) into the annular chambers was kept constant. Each tank was illuminated by a 40 watt incandescent light and the entire assembly, illustrated in Figure 1, was surrounded with black plastic sheets to minimize disturbance.

For the non-exercised trout, white polyethylene tanks (Rosedale Plastics, Montreal) measuring 55.5 cm long, 46 cm wide and 36 cm deep giving a volume of 92-L were used. These tanks were individually surrounded with styrofoam and

the tops were covered with netting and black plastic to minimize outside disturbances. This apparatus was also surrounded by black plastic sheets. These tanks were also supplied with a continuous flow of water at 12°C or 18°C depending on the test and were illuminated by fluorescent lights. Cyanide was metered into these tanks by Marriotte bottle delivery (Leduc 1966a) (Figure 2).

A flow rate of 1 L/min/tank maintained by Manostat predictability flowmeters (Manostat Corp., New York, N.Y.) gave 99% molecular replacement in 6 hours (Sprague 1973) for each bioassay tank. The photoperiod for both sets of bioassay tanks was controlled by a time-switch at 12 h light/ 12 h dark.

Water Chemistry

Certain chemical parameters of the water were measured and/or obtained from the City of Montreal Water Works Department. For the mean values of these measurements see Table 1.

Table 1. Mean values for the chemical parameters of the Montreal city water used during this study.

	SUMMER		WINTER	
	1982	1983	1983	1984
Hardness (mg/L)	128	122	128	130
Alkalinity (as CaCO3 mg/L)	86	79	84	89
Oxygen (% saturation)	95	96	88	91
pH	7.14	7.35	7.28	7.58

The pH was measured during each test with a pH meter, while the oxygen concentration was measured using a YSI model 54A oxygen meter. The cyanide stock solutions were prepared by dissolving KCN reagent in distilled water as done in accordance to Leduc (1966b). Cyanide concentrations were determined once to twice daily, according to the colorimetric method by Lambert et al (1975) on a Bausch and Lomb Spectronic 70 spectrophotometer at 575 nm. The cyanide flows were adjusted when necessary but never differed by more than 5% from the desired values.

Experimental Design

Acute Toxicity Bioassays

The acute toxicity tests were initially designed to compare the 96-h LC50 of HCN between exercised and non-exercised trout at 12°C. The bioassays were carried out following the standard methodology of Sprague (1973). Twelve fish were randomly distributed (random selection via random numbers table) into each bioassay tank and acclimated to the test conditions for two weeks. The mean weights of the fish used for each of these bioassays did not differ between or among the exercised and non-exercised trout. Overall, the trout used for these bioassays had a weight of approximately 12 g. More specific weights and lengths are presented in Tables 3 and 5 of the Results section. The ages of these fish ranged from 10 months to 18 months

depending on the time of year in which the bioassay was carried out. During the acclimation period, the fish were fed a daily maintainance diet of 0.5 to 1% body weight/day with trout chow until 48 h prior to the beginning of the tests. No food was given during the cyanide exposure.

One day before the bioassay began, two fish were removed from each tank to give a total of ten fish per bioassay tank. On the day the bioassays began, the tanks were cleaned of excrement and a calculated amount of cyanide stock solution was mixed into each tank to immediately produce the predicted concentration. This was considered time zero.

Five test concentrations of cyanide and a control were used for each set of bioassays. Generally, the concentrations ranged between 0.036 mg/L to 0.100 mg/L HCN (Appendix 1) and were selected according to preliminary screening bioassays.

Since the exercised trout showed no significant difference in 96-h LC50s at different times of year, it was decided to verify if fish held at a higher metabolic rate due to temperature also exhibited this lack of seasonal change in sensitivity. An additional 96-h LC50 bioassay was carried out at 18°C during late summer (08/83-09/83) for non-exercised fish only, under the same conditions as for the previous bioassays at 12°C for the non-exercised trout. All control groups were maintained under identical test conditions except for the presence of cyanide.

Observations on mortality were made on a logarithmic time scale up to 24 h and every 24 h thereafter until completion of the bioassay at 96 h. To obtain further information on survival times and mortality during the winter (02/03/83 : day/mth/yr) bioassay, observations were maintained up to 192 h. The criteria for death was a lack of response to probing and cessation of gill movement. The dead fish were immediately removed, weighed and total lengths measured.

Acclimation Test

Upon completion of the winter season 96-h LC50 bioassays, it was seen that there was a seasonal influence on the lethal response to cyanide even though all other factors were kept constant. The winter mode fish were received at the laboratory on 02/02/83 and tested on 02/03/83 after 4 w acclimation to laboratory conditions at 12°C and 12 h light-dark photoperiod. The higher sensitivity to cyanide exhibited by the non-exercised trout in the winter may have been due to an inadequate acclimation period. An experiment was designed to verify if a longer acclimation (10 w) to laboratory conditions would change the sensitivity of the fish. The hypothesis was that the fish received during the winter, but acclimated to summer-like conditions for 10 w would be more resistant to cyanide than winter fish acclimated for only 4 w. Fish obtained on 02/02/84 were held in their holding tanks for 8 w and then

acclimated for a further 2 w to experimental conditions giving a total of 10 w at 12°C and 12 h light/ 12 h dark photoperiod. The mean lengths and weights of the fish used for this bioassay at 10 w acclimation were 10.6 cm and 8.10 g. Since there were only 20 - 30 fish available for this test only two concentrations of cyanide were tested under non-exercise conditions. They were similar to two concentrations tested in the previous experiment (02/03/84), 0.040 mg/L HCN and 0.052 mg/L HCN. The observations lasted over 192 h rather than 96 h but all other procedures were as previously described.

Sublethal Toxicity Bioassays

Three sublethal toxicity experiments were carried out during this study, one 20 d growth experiment, and two 15 d thiocyanate bioaccumulation experiments.

Growth Experiment

For the growth experiment, fish of about 11 g were randomly distributed (random numbers table) at a density of 25 fish per tank among four annular tanks and four rectangular tanks and acclimated for two weeks. Before the beginning of the experiment the fish were starved for 24 h. On Day 0, 20 fish per tank were randomly selected, anesthetized in a solution containing 20 mg/L MS222 (tricaine methane sulphonate), blotted dry, weighed,

measured and individually branded with liquid nitrogen (Mighell 1969) before they were returned to their respective tanks. The cyanide stock solutions were then metered into the tanks, except for the controls, to produce sublethal concentrations of 0.005, 0.010 and 0.020 mg/L HCN.

During this experiment, the fish were fed daily a growth ration of 2.5% body weight/day beginning on Day 0. They were fed twice daily and the tanks were cleaned of excrement by siphoning before each feeding. On Day 10 a random sample of 5 fish per tank were killed, weighed and measured. These fish were oven-dried at 80°C for 10 d to obtain individual dry weights. The remaining 15 fish were anesthetized, weighed, measured and returned to their appropriate tanks. The rations were then adjusted accordingly. Termination of the experiment was on Day 20, when all the surviving fish in each tank were killed, weighed and measured. These fish were also oven-dried for dry weight measurements.

Blood Plasma Thiocyanate Accumulation

Two 15 d experiments were carried out with 45 and 72 g rainbow trout. A total of 20 fish per tank were placed in 3 annular and 3 rectangular tanks. One control and two cyanide levels of 0.010 mg/L HCN and 0.020 mg/L HCN were tested. Because a few fish died in the annular tanks during the acclimation period in Experiment 1 (02/09/83), a total of three fish per tank were taken and sacrificed as

pre-exposure (prior to cyanide exposure) samples while for Experiment 2 (23/01/84) a total of 5 fish were sampled on Day 0. The remaining sample dates were on Days 2, 7 and 15, when 5 fish per tank were sampled. During the two experiments, the fish were fed a daily ration of 1.6% body weight/day but no food was given 24 h prior to each sampling date. Before each feeding, the tanks were cleaned of excrement by siphoning. The fish were killed with a blow on the head, weighed, measured and sampled for blood. Blood was sampled by severing the caudal peduncle and collecting the blood into syringes rinsed with a 20% solution of sodium citrate, an anticoagulant. The blood sample was then emptied into 1.5 ml Eppendorf microtubes which were then kept on ice. The blood samples were centrifuged and the plasma was then transfered to clean Eppendorf microtubes and stored on ice in a refrigerator for later determination of thiocyanate content. All determinations were completed within 36 h after sampling. To accumulate an adequate volume of blood plasma for thiocyanate analysis, samples were pooled. From the 5 samples, I prepared 2 samples, each of these contained the total blood sampled from 2 fish plus 1/2 of the sample from the remaining fish. This was done on all sample days except Day 0 for Experiment 1. Readings for Day 0 of Experiment 1 were the result of pooling 3 fish into 2 samples, so the total sample was from 1 fish plus 1/2 of the remaining fish.

The blood plasma thiocyanate levels were measured by

the modified Lambert et al (1975) method (Raymond 1984). A 10% solution of trichloroacetic acid (TCA) was added in a 10 to 1 proportion to the plasma samples to precipitate any proteins when centrifuged. One ml of the above supernatant was mixed with one ml of phosphate buffer (NaH_2PO_4 and K_2HPO_4) of pH 7.90 to raise the pH of the plasma/TCA solution to 6.0 before assaying for thiocyanate. The thiocyanate was measured colorimetrically (Lambert et al 1975). All measurements were in duplicate.

Liver Glycogen

Liver glycogen was measured on fish sampled on Days 0, 7 and 15 of the thiocyanate Experiment 2 previously described. Immediately after the fish were killed, the livers were removed and frozen in liquid nitrogen and stored in a freezer at -10°C. All glycogen determinations were made within 5 d of sampling. Frozen whole livers were transferred to test tubes and dissolved in 30% KOH (2 ml per g of tissue). Liver weights ranged from 0.50 to 1.40 g. The test tubes were then immersed in boiling water for 10 to 15 min and when the tissues were fully dissolved, 1.1 to 1.2 volumes of 95% ethanol were added to the test tubes and centrifuged for 25 min (Good et al 1933). The supernantant was decanted, the remaining pellet was dissolved in distilled water and diluted to a known volume (approximately 10 -15 ml). From this unknown glycogen solution, 2 ml aliquots were transferred to colorimetric tubes where 0.1 ml

of 80% phenol and 5 ml of concentrated reagent grade sulfuric acid were added in that order. The colorimetric tubes were then incubated for 60 min at room temperature. This phenol-sulphuric colorimetric method of Montgomery (1957) was done in duplicate to determine the glycogen concentration using a Bausch and Lomb Spectronic 70 spectrophotometer at 490nm. A standard curve of known glycogen concentrations was developed in a similar manner.

Statistical Methods

The 96-h LC50 values, slope function and 95% confidence intervals were calculated from log-probit paper following the nomographic methods of Litchfield and Wilcoxon (1949). The median survival times (MST) and 95% confidence intervals were determined as described by Litchfield (1949).

Since the growth and thiocyanate studies tested two factors simultaneously, cyanide and exercise or thiocyanate accumulation and exercise, two-way analyses of variance (ANOVA) was used (Sheffler 1979, Sokal and Rohlf 1981). One of the assumptions of the ANOVA is that the data being tested must have similar variances (homogeneity). To test homogeneity, an Fmax test was used. If a significant Fmax was obtained (heterogeneous variances) the data were log transformed. When significant F values were obtained a Least Significant Difference (L.S.D.) test was used to determine which treatment groups were significantly different. To investigate the separate influence that

exercise has on growth, the mean growth rates of exercised and non-exercised control fish were compared using a Student's t-test.

Analysis of the liver glycogen data was the same as that used in the growth and thiocyanate bioaccumulation experiments. However, to obtain more information on the influence of exercise on glycogen, the data from the exercised trout were pooled and compared with the pooled data from the non-exercised trout on Day 0. This data was analysed for differences in glycogen reserves due to exercise using a Student's t-test. The two-way ANOVA was then used on the data from Day 7 and Day 15 to analyse the influence of cyanide and exercise on liver glycogen levels.

RESULTS

Acute Toxicity Tests

Results of the acute toxicity (96-h LC50) tests of cyanide on exercised and non-exercised trout and at different times of the year at 12°C are presented in Tables 2, and 3 and in Figure 3.

The first results obtained (03/08/82, see Table 2) suggested that cyanide concentrations tested were too low to cause any significant mortality and that the trout were more resistant than those tested by Kovacs and Leduc (1982a), (96-h LC50) 0.042 mg/L HCN at the same temperature, but in the winter (20/12/78 Kovacs pers.comm.). These results also show that there was little to no difference in toxicity between exercised and non-exercised trout. The mortality values from Table 2 were then all combined and a 96-h LC50 of 0.058 mg/L HCN was estimated. The hypothesis of varying sensitivity due to seasonal influence was then added to the hypothesis of increased resistance due to exercise on cyanide toxicity. The data of all further acute toxicity tests presented in Table 3 and illustrated in Figure 3 show that, at 12°C, exercise significantly elevates the LC50 value only during the winter; during the spring and summer there is no significant difference between exercised and non-exercised trout but the later remain slightly more sensitive by a factor of 3.5 - 5%. It is also clear from Figure 3 that cyanide toxicity varies seasonally, with this

phenomenon being more pronounced in the non-exercised trout. During late summer (24/09/82), the non-exercised trout had a 96-h LC50 value of 0.055 mg/L HCN which during the winter, dropped by more than 20% ($p < 0.05$) to a value of 0.043 mg/L HCN. This response was not as pronounced in the exercised trout, as the reduction in the 96-h LC50 value from summer to winter was about 9% (not significant).

Table 2. Results of a preliminary acute toxicity test showing mortality after 96h at various cyanide concentrations among exercised and non-exercised rainbow trout at 12°C in the summer (03/08/82).

	HCN (mg/L)	Percent Mortality
Exercised	0.000	0
	0.026	0
	0.032	0
	0.038	0
	0.047	0
	0.071	100
Non-Exercised	0.000	0
	0.030	0
	0.035	0
	0.040	0
	0.048	0
	0.064	80

Along with the 96-h LC50 values, median survival times (MST) were also calculated. The median survival time values give us more insight as to what is happening to each group of fish. The MST values were used to construct a toxicity curve (Figure 4) for summer and winter testing periods;

Figure 3. Seasonal variations in the 96-h LC50 value for hydrogen cyanide in exercised and non-exercised rainbow trout at 12°C and non-exercised rainbow trout at 18°C. Solid circle and square are values from Kovacs and Leduc (1982a).

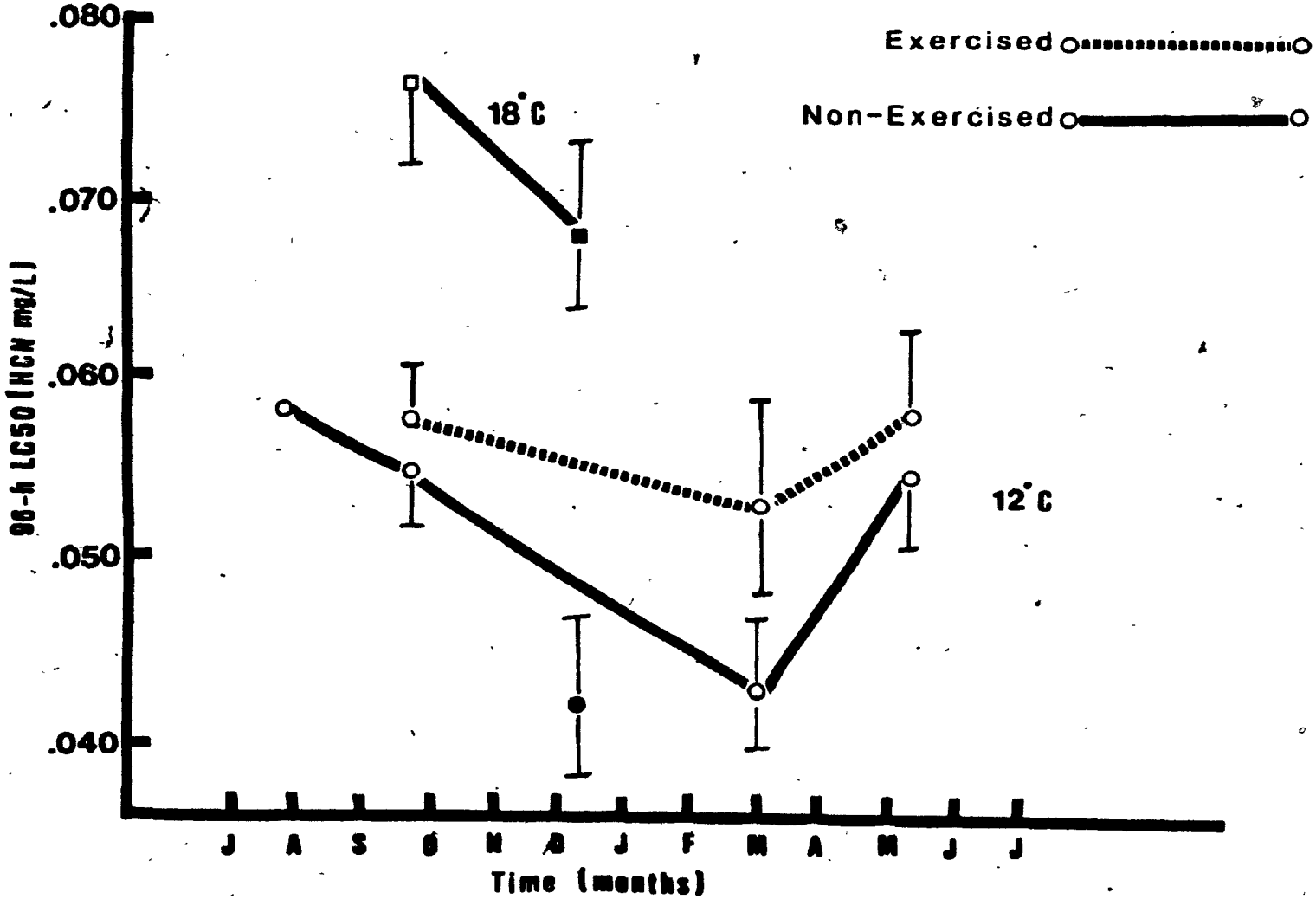

.080
.070
.060
.050
.040
96-h LC50 (HCN mg/L)
Exercised
Non-Exercised
18°C
12°C
J A S O N D J F M A M J J
Time (months)

Table 3. Acute toxicity of hydrogen cyanide to exercised (12.1 cm/s) and non-exercised rainbow trout at 12°C at different times of the year, showing the 96-h LC50 with other biological parameters.

Date of Experiment	24/09/82 (30/08/82)[a]		02/03/83 (02/02/83)[a]		12/05/83 (24/04/83)[a]	
Season	Late Summer		Winter		Spring	
	Exercised	Non-Exercised	Exercised	Non-Exercised	Exercised	Non-Exercised
96-h LC50 and 95% confidence interval (mg/L HCN)	0.057 (0.055-0.060)	0.055 (0.051-0.059)	0.052 (0.047-0.058)	0.043 (0.040-0.046)	0.058 (0.054-0.062)	0.055 (0.051-0.059)
Slope function	1.06	1.08	1.12	1.11	1.08	1.08
Highest conc. with no mortality in 96 hrs (mg/L HCN)	0.045	0.044	0.038	0.033	0.042	0.040
Lowest conc. causing 100% mortality in 96 hrs (mg/L HCN)	0.062	0.064	0.073	0.062	0.061	0.066
Mean weight of fish and range (g)	15.75 (11.62-21.50)	15.32 (10.43-22.25)	10.22 (8.15-13.23)	10.13 (7.20-13.33)	10.45 (7.60-16.00)	11.59 (8.07-14.96)
Mean total length of fish and range (cm)	11.5 (10.0-12.7)	11.4 (9.6-12.7)	9.9 (9.2-10.9)	9.9 (9.2-11.4)	10.6 (9.7-11.6)	10.9 (9.7-12.1)

[a] Date trout arrived to laboratory from hatchery

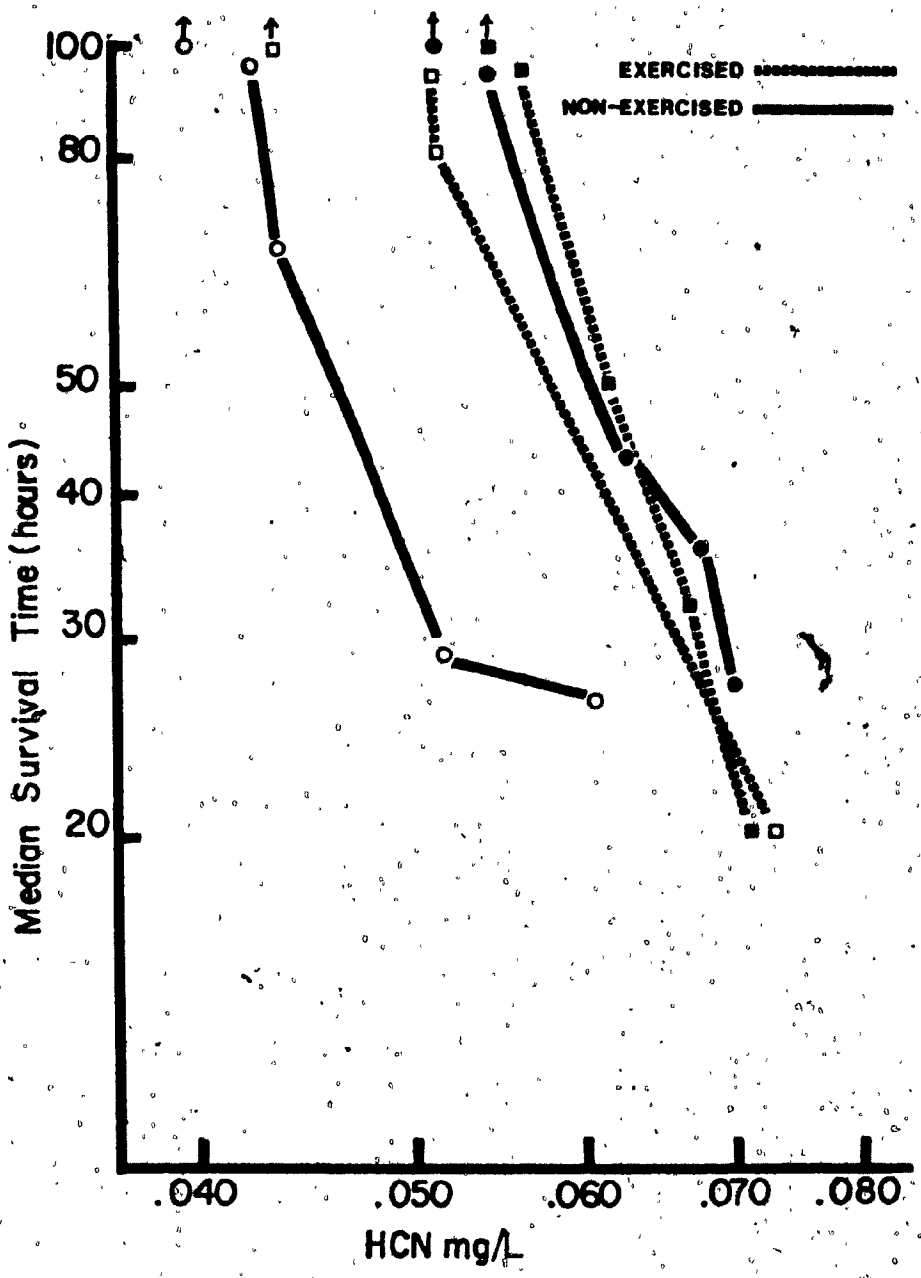
however only data from comparable cyanide concentrations were examined statistically and they are presented in Table 4. All of the MST values for these bioassays are preserved in Appendix 1. The median survival times from Table 4 and Figure 4 show that survival times between the exercised and non-exercised trout during the summer do not differ significantly ($p > 0.05$). However, during the winter

Table 4. Median survival times (MST), 95% confidence intervals and percent mortality of exercised and non-exercised rainbow trout exposed to various concentrations of cyanide at 12°C during different seasons throughtout the year.

Season/ Treatment	HCN (mg/L)	MST (hours)	confidence interval	% Mortality at 96h
Late Summer				
Exercised	0.045	>96	---	0
Non-Exercised	0.044	>96	---	10
Exercised	0.062	50	(45 - 55)	100
Non-Exercised	0.063	42b	(36 - 50)	100
Exercised	0.071	20	(16 - 25)	100
Non-Exercised	0.070	26	(22 - 30)	100
Winter				
Exercised	0.044	>96	---	20
Non-Exercised	0.044	66	(57 - 76)	90
Exercised	0.053	79a	(46 - 134)	50
Non-Exercised	0.052	29a	(23 - 35)	90
Non-Exercised	0.062	27b	(24 - 30)	100

common subscript for values significantly different [$p < 0.05$, Litchfield (1949)]

Figure 4. Toxicity curves of hydrogen cyanide for exercised and non-exercisede rainbow trout at 12°C during the summer (solid symbols) and winter (open symbols).


EXERCISED
NON-EXERCISED
Median Survival Time (hours)
100
80
50
40
30
20
.040
.050
.060
.070
.080
HCN mg/L

at 0.044 and 0.052 mg/L HCN, the non-exercised trout show a significant ($p < 0.05$) decrease in survival times by as much as 63% from the MST's of exercised trout. The non-exercised trout at 0.062 mg/L HCN showed a 36% decrease (significant $p < 0.05$) in survival time from summer to winter testing while the exercised trout maintain the same survival times they had in the summer. Similar findings were observed at 0.044 mg/L HCN. Thus, for any given concentration, non-exercised trout in the winter have a shorter median survival time than those tested in the summer (Figure 4) when exposed to the same concentration of cyanide, thus reinforcing previous findings of the 96-h LC50 values on seasonal changes in sensitivity. The curves from Figure 4 also show that in the low concentration range of 0.040 - 0.060 mg/L HCN, the non-exercised trout have shorter survival times than the exercised ones; this difference in survival times is reduced as the concentration of cyanide increases above 0.060 mg/L HCN.

Since exercised trout showed relatively little seasonal variation in cyanide sensitivity (Figure 3) compared with the non-exercised trout, it was then hypothesised that non-exercised fish maintained at a higher metabolic rate due to higher water temperature, would show little seasonal variation in cyanide sensitivity. This hypothesis was tested with non-exercised trout during the summer (08/83-09/83) at 12°C and 18°C and the results were compared with those of Kovacs and Leduc (1982a) who tested acute

toxicity at 12 and 18°C during the winter (20/12/78). The work carried out by Kovacs and Leduc (1982a) was completed in the same laboratory, using the same sized trout and under similar conditions as this study. They found the winter 96-h LC50 to be 0.042 mg/L and 0.068 mg/L HCN at 12 and 18°C respectively. This comparison is shown in Table 5 and Figure 3. The 9% reduction in the 96-h LC50 value from "summer" fish to "winter" fish at 18°C is not significant ($p > 0.05$) while a reduction of 30% at 12°C is ($p < 0.05$). Although no additional information was gained from the median survival times, they are presented in Appendix 2.

To further assess the seasonal change in fish sensitiviy to cyanide, an acclimation experiment was conducted. It was hypothesised that by extending the laboratory acclimation period, a reduction in the seasonal response would be observed. The results of this experiment are presented in Table 6. It appears that a longer acclimation period during the winter affects trout survival time when exposed to cyanide. It was observed that by extending the acclimatory period from 4 weeks to 10 weeks MST were increased by about 82% (significant $p < 0.05$, Litchfield (1949) method). These survival times are still less than those found in the Spring.

Table 5. Comparison of acute toxicity of hydrogen cyanide to non-exercised rainbow trout acclimated and tested at different temperatures during summer and winter showing the 96-h LC50 with other biological parameters.

Date of Experiment	TEMPERATURE 12°C 09/08/83	12°C 20/12/78[a]	18°C 21/09/83	18°C 20/12/78[a]
96-h LC50 and 95% confidence interval (mg/L HCN)	0.058* (0.055 - 0.061)	0.042* (0.038 - 0.046)	0.076 (0.071 - 0.081)	0.068 (0.064 - 0.072)
Slope function	1.09	1.30	1.07	1.12
Highest conc. with no mortality in 96 hrs (mg/L HCN)	0.045	0.032	0.065	0.060
Lowest conc. causing 100% mortality in 96 hrs (mg/L HCN)	0.070	0.053	0.100	0.087
Mean weight of fish and range (g)	14.14 (11.04 - 18.53)	12.41 (8.29 - 16.02)	13.98 (8.94 - 22.52)	13.19 (9.63 - 17.21)
Mean length of fish and range (cm)	11.5 (10.7 - 12.7)	10.2 (9.0 - 11.1)	11.4 (10.1 - 13.7)	10.6 (9.5 - 11.9)

[a] Data from Kovacs and Leduc, 1982
* Significantly different ($p < 0.05$)

Table 6. Comparison of lethal response results of non-exercised trout exposed to cyanide after different acclimation periods to laboratory conditions during the winter and exposure during the spring at 12°C.

Test Condition	HCN (mg/L)	MST (hours)	95% confidence interval	%Mortality at 96h
4 weeks Acclimation	0.040	105	72 - 153	40
	0.052	29	23 - 35	90
10 weeks Acclimation	0.040	192	128 - 286	10
	0.052	53	44 - 63	80
Spring * 12/05/83	0.040	>96	---	0
	0.052	>96	---	30

MST - median survial time
* 4 weeks acclimation

Sublethal Toxicity Studies

There were three experiments carried out to compare the sublethal toxicity of cyanide to exercised and non-exercised trout: a 20-day growth experiment and two 15-day thiocyanate bioaccumulation multiple sample experiments.

Growth Experiment

Since some toxicants may alter water uptake by the fish, a comparison of dry and wet weights was done on samples of Days 10 and 20 of the cyanide growth experiment and Days 0 and 15 of the second thiocyanate experiment. A two-way factorial analysis of variance (ANOVA) (Sheffler 1979) compared the water content of exercised and non-exercised trout exposed to different cyanide concentrations. The results of these tests revealed that there was no alteration in the body water content (Table 7). Therefore individual growth rates were expressed as the means of specific growth rates (MSGR) in percent gain per day wet weight, calculated from Brown's (1957) equation:

$$MSGR = 100 \times \frac{\ln YT - \ln Yt}{T - t}$$

where lnYT and lnYt are the natural logarithms of the wet weight of fish at time T and t days. The mean specific growth rates for the exercised and non-exercised cyanide-exposed trout were compared with those of the controls.

The growth rates (MSGR) of exercised and non-exercised rainbow trout during the 0-10, 10-20 and 0-20 d periods, are

Table 7. Comparison of mean percent body water content in exercised and non-exercised rainbow trout exposed to various concentrations of cyanide at 12°C.

(a) 20-day exposure - growth experiment

	HCN (mg/L)	% water: Day 10	% water: Day 20
Exercised	0.000	78.06	76.40
	0.005	78.06	75.58
	0.010	77.17	76.62
	0.020	77.14	76.24
Non-Exercised	0.000	77.74	76.12
	0.005	76.95	75.71
	0.010	75.74	76.14
	0.020	76.76	75.98

(b) 15-day exposure - thiocyanate experiment #2

	HCN (mg/L)	% water: Day 0	% water: Day 15
Exercised	0.000	76.60	75.62
	0.010	75.85	76.55
	0.020	75.62	*
Non-Exercised	0.000	76.34	75.11
	0.010	75.13	74.76
	0.020	76.17	74.62

* fish mortality due to high cyanide conc. on Day 9.

presented in Table 8 and Figure 5. Over the 20-d period, cyanide affected growth of exercised and non-exercised fish differently; the growth of exercised fish was clearly stimulated by cyanide at all concentrations while the non-exercised fish had their growth depressed only at the highest concentration while at the lower concentrations growth was unaffected.

Throughout the 20-d growth experiment, the control exercised trout grew less than the non-exercised controls. However, it was not until Day 20 that this reduction became significant ($F= 2.99$, $df3,101$; $p < 0.05$).

As illustrated in Figure 5, responses in growth during the first 10-d period were irregular. The exercised trout at the two lowest cyanide concentrations exhibited slightly higher growth rates while trout at the highest concentration (0.020 mg/L HCN), exhibited a 7% reduction. The non-exercised trout showed slightly lower growth rates at 0.005 mg/L HCN (10%) and a significant ($F= 6.06$, $df3,147$; $p < 0.05$) reduction at 0.020 mg/L HCN (28%).

During the following 10 d, all cyanide-exposed exercised trout grew faster than the controls, however trout exposed to 0.010 mg/L HCN did not grow as fast as they did during the first 10 days. The non-exercised trout at the two lowest cyanide concentrations grew as well as their controls during the 10-20 d period while the trout exposed to 0.020 mg/L HCN still grew slightly slower (not significant). To better compare the growth rates between

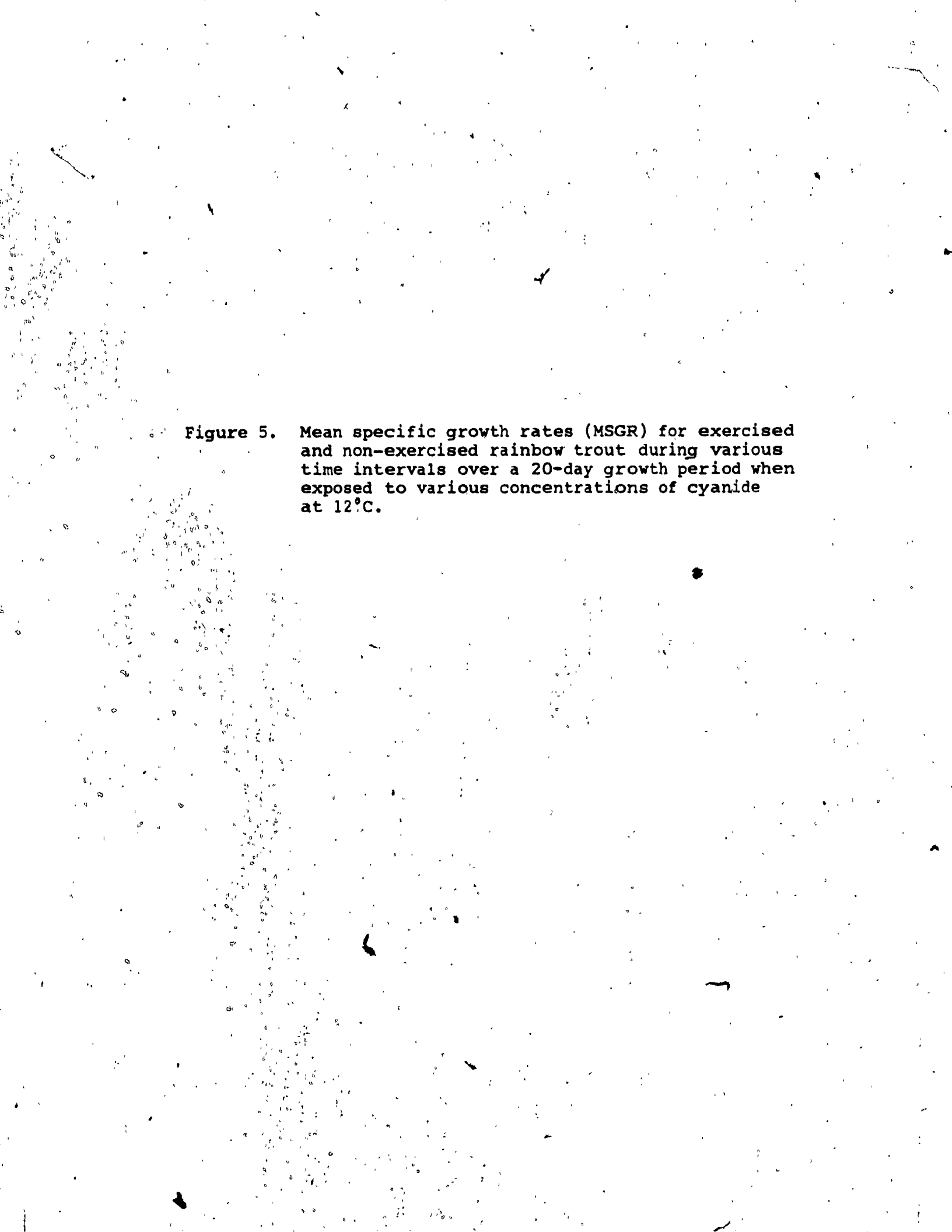


Figure 5. Mean specific growth rates (MSGR) for exercised and non-exercised rainbow trout during various time intervals over a 20-day growth period when exposed to various concentrations of cyanide at 12°C.

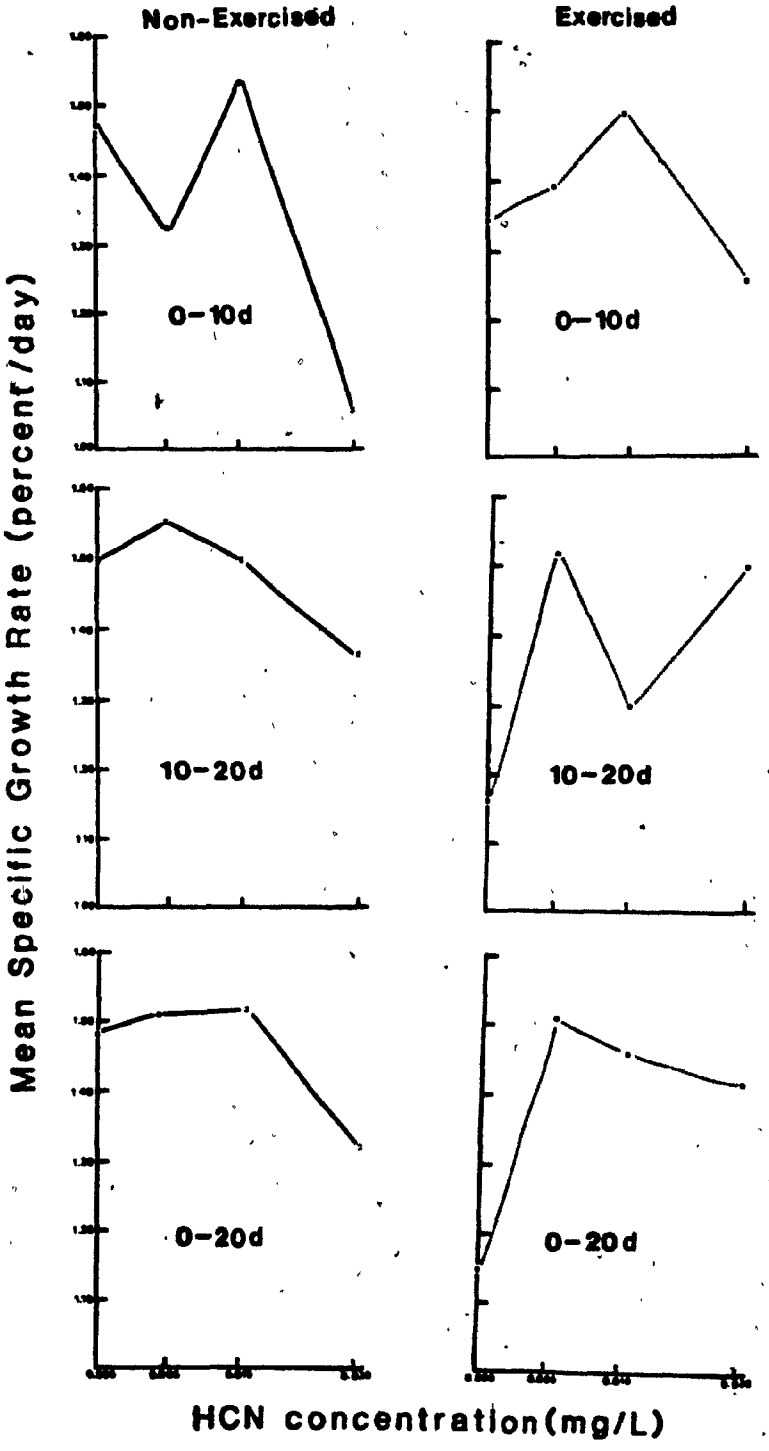

Non-Exercised
Exercised
0–10d
0–10d
10–20d
10–20d
0–20d
0–20d
Mean Specific Growth Rate (percent/day)
HCN concentration(mg/L)

Table 8. Results of the growth experiment comparing exercised and non-exercised rainbow trout exposed to various concentrations of cyanide during a 20-d period at 12°C showing the initial wet weights and mean specific growth rate (MSGR) in wet weight at different intervals during the experiment.

Treatment	Cyanide conc. (mg/L HCN)	Initial mean wet weight (g)	MSGR: 0-10 (S.D.) (%/d)	n	MSGR: 10-20 (S.D.) (%/d)	n*	MSGR: 0-20 (S.D.) (%/d)	% change of MSGR from 0-10 to 10-20d
Exercised	0.000	11.30	1.356 (0.448)	20	1.160 (0.461)	12	1.149 (0.333)	-15
	0.005	11.80	1.395 (0.334)	18	1.517 (0.537)	14	1.515 (0.233)	+8
	0.010	10.60	1.496 (0.423)	20	1.302 (0.308)	15	1.474 (0.212)	-12
	0.020	10.90	1.266 (0.388)	20	1.506 (0.321)	15	1.426 (0.276)	+19
Non-Exercised	0.000	10.99	1.471 (0.267)	20	1.503 (0.259)	12	1.476 (0.269)	+2
	0.005	10.70	1.318 (0.328)	19	1.558 (0.337)	13	1.503 (0.290)	+18
	0.010	10.80	1.542 (0.266)	20	1.501 (0.437)	15	1.509 (0.286)	-4
	0.020	10.40	1.055 (0.483)	18	1.377 (0.500)	13	1.318 (0.321)	+31

S.D. - standard deviation
*n** - same sample size for 10-20 d period and 0-20 d period

the 1st and the 2nd 10-d period, percent changes in growth rates were calculated and presented in Table 8. It can be seen that the growth responses are mostly altered at 0.020 mg/L HCN. During the first 10 days of exposure to this concentration (0.020 mg/L HCN), growth was reduced in both exercised and non-exercised trout, but during the following 10 days, growth was increased by 18% and 31%. This 31% increase exhibited by the non-exercised trout at 0.020 mg/L HCN was significant ($F= 3.97$, $df1,122$; $p < 0.05$).

Looking at the overall 20-day growth period (Figure 5), the results for the exercised trout indicate that all three concentrations of cyanide enhanced growth by as much as 32%, with this enhancement being significant ($F= 2.99$, $df3,101$; $p < 0.05$) at 0.005 and 0.010 mg/L HCN. However, by Day 20, the non-exercised trout showed little influence of cyanide because of the growth rebound during the 10 - 20 d period at 0.005 and 0.020 mg/L HCN. Overall, cyanide had no effect on growth in non-exercised trout, but it boosted growth in exercised trout by as much as 32% above their controls. Because of this elevation in growth in the cyanide-exposed exercised trout, growth rates of these exercised trout were similar to the non-exercised trout exposed to cyanide.

Blood Plasma Thiocyanate Accumulation

Two 15-day experiments were carried out to compare the accumulation of thiocyanate (SCN^-) in blood plasma of

exercised and non-exercised trout during exposure to cyanide. Experiment 1 was completed in early September with rainbow trout of mean weight of 45 g and Experiment 2 was carried out in January with trout weighing 72 g.

The results given in Tables 9 and 10 and graphically illustrated in Figure 6 show that the basal levels of blood plasma thiocyanate in control fish differed somewhat between the two experiments but remained stable and much lower than in the cyanide-exposed fish. The levels of thiocyanate in the exposed trout increased with the duration of exposure to cyanide for both exercised and non-exercised trout.

The results shown in Figure 6 clearly show that by Day 2, the thiocyanate levels are significantly elevated (Exp.1 $F= 48.24$, $df2,16$; $p < 0.05$; Exp.2 $F= 52.62$, $df2,16$; $p < 0.05$) in cyanide-exposed trout (0.010 mg/L and 0.020 mg/L HCN) but do not differ between the two concentrations of cyanide tested.

In Experiment 1 (Table 9), thiocyanate levels maintained a similar pattern of accumulation for the exercised and non-exercised trout at all levels of cyanide until Day 7 when the non-exercised trout appear to have reached their maximum in thiocyanate accumulation. By Day 15 the non-exercised trout showed no increases of thiocyanate levels from Day 7 ($F= 0.425$, $df1,18$; $p > 0.20$). The exercised trout differed slightly since, at the lowest cyanide concentration (0.010 mg/L HCN) there was a lag in thyocyanate build-up as the thiocyanate levels increased

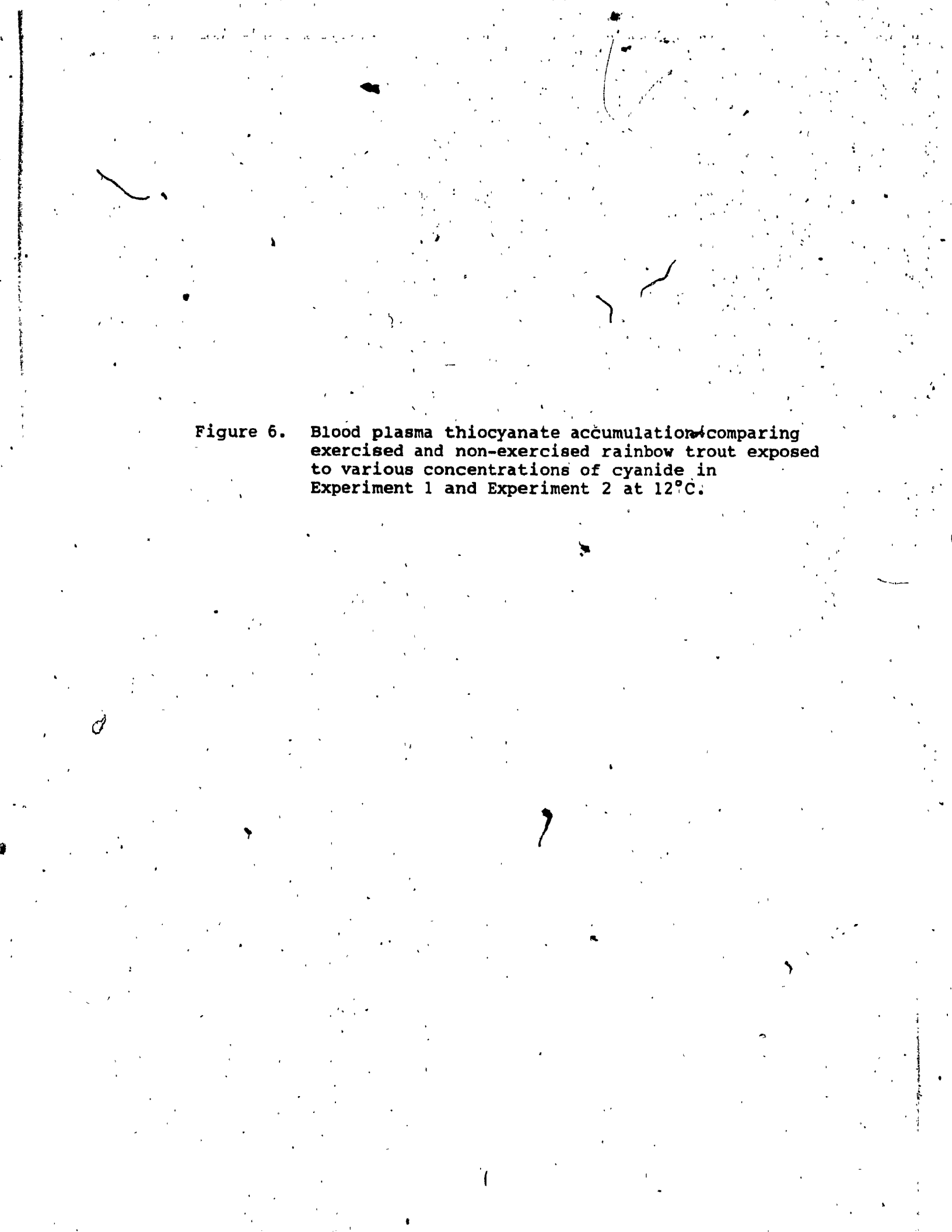


Figure 6. Blood plasma thiocyanate accumulation comparing exercised and non-exercised rainbow trout exposed to various concentrations of cyanide in Experiment 1 and Experiment 2 at 12°C.

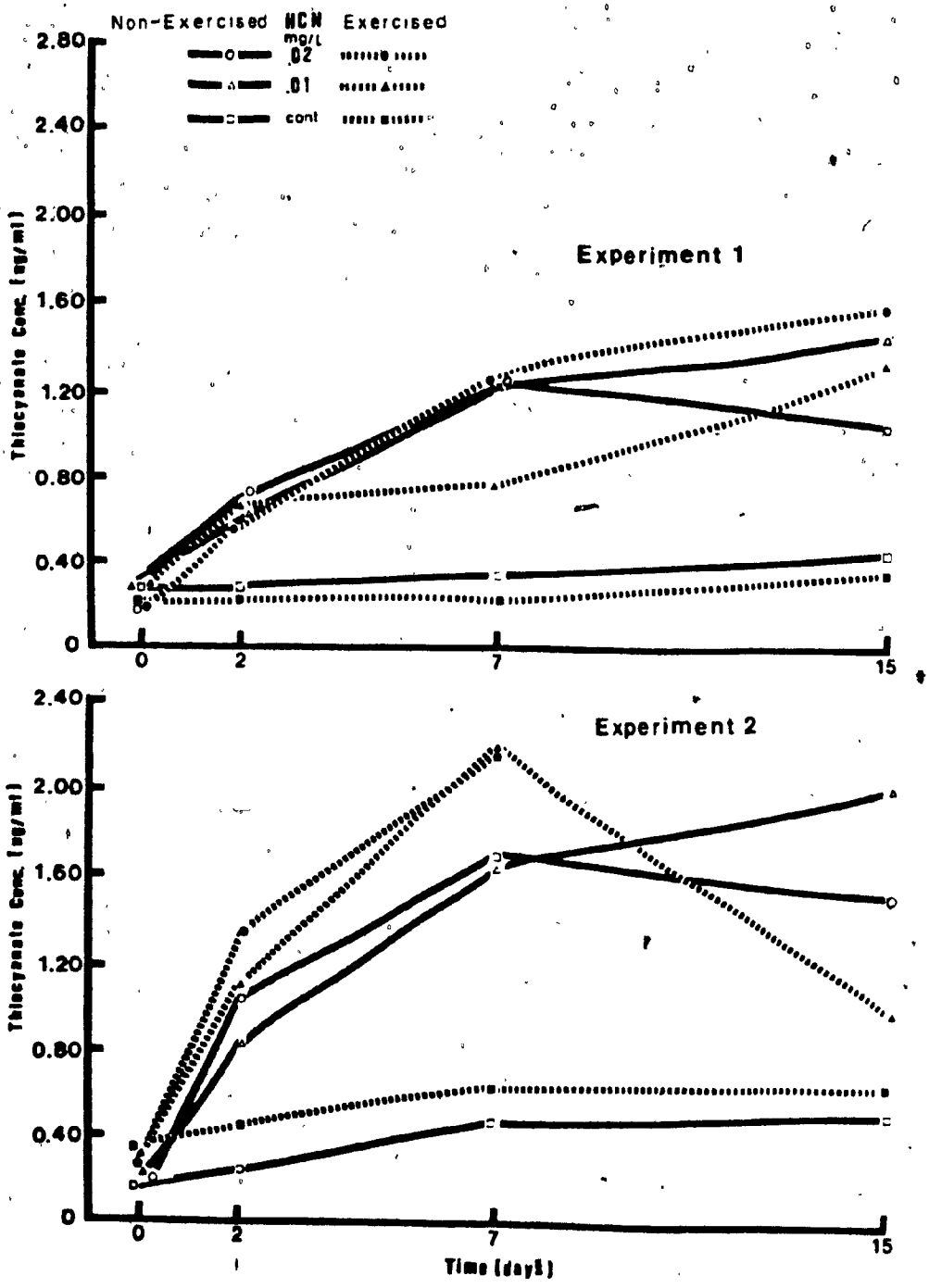

Non-Exercised HCN mg/l Exercised
.02
.01
cont
Experiment 1
Thiocyanate Conc. (mg/ml)
2.80
2.40
2.00
1.60
1.20
0.80
0.40
0
0
2
7
15
Experiment 2
Thiocyanate Conc. (mg/ml)
2.40
2.00
1.60
1.20
0.80
0.40
0
0
2
7
15
Time (days)

Table 9. Mean blood plasma thiocyanate levels in exercised and non-exercised rainbow trout (45 g) exposed for 15 days to various concentrations of cyanide at 12°C (experiment 1).

Day of Exposure	Cyanide conc. (mg/L HCN)	Exercised Sample size	Exercised Mean plasma SCN^- (ug/ml)	Exercised (S.D.)	Non-Exercised Sample size	Non-Exercised Mean plasma SCN^- (ug/ml)	Non-Exercised (S.D.)
0	0.000	5	0.2195	0.0361	5	0.2610	0.0354
	0.010	5	0.2451	0.0361	5	0.2780	0.0310
	0.020	5	0.1790	0.0410	5	0.1302	0.0542
2	0.000	5	0.2334	0.0718	5	0.2680	0.0310
	0.010	5	0.6424	0.0479	5	0.6335	0.1153
	0.020	5	0.5035	0.0992	5	0.6950	0.1575
7	0.000	5	0.2171	0.0952	5	0.3537	0.0133
	0.010	5	0.7336	0.3888	5	1.2333	0.1311
	0.020	5	1.2597	0.3306	5	1.2535	0.1472
15	0.000	5	0.3866	[illegible]	5	0.4672	0.1367
	0.010	5	1.2889	0.1355	5	1.4317	0.3836
	0.020	5	1.5620	0.1128	5	1.005	0.0657

S.D. - standard deviation

Table 10. Mean blood plasma thiocyanate levels in exercised and non-exercised rainbow trout (72 g) exposed for 15 days to various concentrations of cyanide at 12°C (Experiment 2).

Day of Exposure	Cyanide conc. (mg/L HCN)	Exercised Sample size	Exercised Mean plasma SCN^- (ug/ml)	Exercised (S.D.)	Non-Exercised Sample size	Non-Exercised Mean plasma SCN^- (ug/ml)	Non-Exercised (S.D.)
0	0.000	5	0.3904	0.0724	5	0.2637	0.0412
	0.010	5	0.3783	0.0575	5	0.3361	0.0304
	0.020	5	0.3663	0.0441	5	0.2825	0.0465
2	0.000	5	0.4721	0.1729	5	0.3511	0.1318
	0.010	5	1.1249	0.0607	5	0.8472	0.0884
	0.020	5	1.3250	0.1288	5	1.0408	0.1296
7	0.000	5	0.6827	0.1293	5	0.4924	0.0303
	0.010	5	2.2367	0.0665	5	1.6216	0.0884
	0.020	5	2.1732	0.4742	5	1.6939	0.1760
15	0.000	5	0.6948	0.0586	5	0.4733	0.0738
	0.010	5	0.9903	0.1035	5	2.0001	0.2767
	0.020	5	[a]		5	1.5442	0.0402

S.D. - standard deviation
[a] At day 9, 3 fish died due to accidental poisoning by cyanide

from Day 7 to Day 15 ($F= 16.03$, $df1,18$; $p < 0.05$). Overall the general trend in Experiment 1 was to accumulate thiocyanate in the blood plasma for 7 to 15 days, with the levels having a tendency to peak and/or level off between Days 7 and 15.

The results of Experiment 2 (Table 10) were similar to those of Experiment 1. Blood plasma thiocyanate levels were significantly ($F= 52.62$, $df1,17$; $p < 0.05$) higher in cyanide-exposed trout by Day 2 and remained high throughout the 15 days. During this experiment there was a distinct difference in accumulation between exercised and non-exercised rainbow trout. By Day 2, the exercised trout at both cyanide concentrations exhibited slightly higher blood plasma thiocyanate levels than the non-exercised trout and by Day 7 the exercised trout showed significantly ($F= 29.21$, $df1,18$; $p < 0.05$) higher thiocyanate levels at both cyanide concentrations. Thiocyanate levels in non-exercised trout kept increasing but at a slower rate. As shown in Figure 6, thiocyanate levels in exercised trout accumulated faster and peaked higher than the non-exercised ones. Unfortunately three out of five remaining exercised trout at 0.020 mg/L HCN died accidently on Day 9 during a switching of the cyanide stock solution bottles, so no data is available on Day 15 for the 0.020 mg/L HCN exercised trout. Because these fish died, a Student t-test was performed on the data from the exercised trout. This analysis reveals that exercised trout at 0.010 mg/L HCN show

a significant ($t= 15.01$ df6; $p < 0.05$) drop in thiocyanate from Day 7 to 15, while the thiocyanate levels in the non-exercised trout remained the same from Day 7 to Day 15 ($F= 0.30$, df1,18; $p > 0.05$).

Liver Glycogen

Liver glycogen was measured on Days 0, 7 and 15 during the second thiocyanate bioassay. The data presented in Table 11 suggest higher levels of liver glycogen in exercised trout than in non-exercised ones on Day 0 but a t-test revealed no significant ($t= 1.66$, df28; $p > 0.05$) differences. Glycogen levels between the exercised and non-exercised trout on Day 7 not significantly different (Day 7; $F= 0.209$, df2,23; $p > 0.25$).

By Day 15 the glycogen reserves appeared to have increased (not significant) in the non-exercised control fish and at 0.010 mg/L HCN while the 0.020 mg/L HCN group remained depressed. Since three trout accidently died on Day 9 in the exercised 0.020 mg/L HCN group, no glycogen measurement was done for Day 15. However, the Day 15 measurement for the remaining two exercise groups show that their glycogen levels remain fairly constant and are the same as the non-exercise fish (Day 15; $F= 0.196$, df1,13; $p > 0.25$). Overall, there appears to have been no influence of cyanide or exercise on liver glycogen reserves in the trout tested.

Table 11. Mean liver glycogen levels in exercised and non-exercised rainbow trout during a 15 day exposure to various concentrations of cyanide at 12°C.

Day of Exposure	Cyanide conc. (mg/L HCN)	Exercised Mean liver glycogen (mg/g) (S.D.)	Non-Exercised Mean liver glycogen (mg/g) (S.D.)
0	0.000	40.98 (12.19)	35.66 (17.26)
	0.010	42.43 (10.14)	35.39 (2.67)
	0.020	39.51 (8.15)	37.84 (8.07)
7	0.000	40.71 (17.55)	31.46 (8.46)
	0.010	45.86 (15.36)	30.32 (11.72)
	0.020	42.31 (4.09)	37.63 (16.37)
15	0.000	50.07 (5.30)	45.43 (7.23)
	0.010	42.55 (1.53)	46.72 (21.90)
	0.020	a	29.19 (11.70)

S.D. Standard deviation
a At day 9, 3 fish died due to accidental poisoning by cyanide

DISCUSSION

Influence of Exercise

Analysis of the 96-h LC50 and median survival times (MST) indicates that the exercised rainbow trout (Salmo gairdneri) are more resistant than the non-exercised ones to lethal concentrations of cyanide. This difference is however much greater during the winter period as the exercised trout appear more independent of the seasonal influence.

Because of the differences in size, shape and lighting, between the bioassay aquaria used for the exercised and non-exercised fish, some of the responses to cyanide attributed to exercise may have been due to the different test conditions, not exercise per se. Several lines of evidence suggest this is not the case. If the different apparatus were to modify the results, similar 96-h LC50 and slope values during the summer and spring tests (Table 3) would not have been observed thus indicating some sort of continuity between the experimental apparatus. Otherwise, the two types of tanks were treated identically (ie.stock of trout, volume and quality of water, food).

The higher resistance exhibited by the exercised trout is probably due to many factors, but is most likely due to their increased metabolic rate which has been shown in many circumstances to have an influence in cyanide toxicity. Smith et al (1978) and Kovacs and Leduc (1982a) found

cyanide toxicity to be inversely related to temperature, higher temperature increases resistance to cyanide. Work by Broderius (1973) also supports the hypothesis that lower metabolic rates increase sensitivity to cyanide. The resistance of stickleback (Gasterosteus sp.) to cyanide was greatly reduced when they were transferred and tested in saltwater (17‰) as compared to those in freshwater. This euryhaline fish has a higher respiratory rate in freshwater than in isosmotic brackish water (Hill, 1976). Sockeye salmon (Oncorhynchus nerka) and rainbow trout also show decreases in metabolic rate when passing from fresh to salt water (Rao 1968, Brett 1971).

The metabolic rate of fish is also size related with smaller fish having a higher specific metabolic rate in oxygen consumption (Beamish 1964a). Studies by Anderson and Weber (1975) and McCracken and Leduc (1980) conclude that smaller fish are more resistant to cyanide. An earlier study by Herbert and Merkens (1952) found that larger (15 - 17 cm) rainbow trout die much sooner in cyanide solutions than do smaller (5 - 7 cm) ones. The survival time for the smaller trout were more than twice as long as those of the larger trout.

This study reveals that resistance to cyanide toxicity in exercised and non-exercised trout may be concentration dependent. The results in Table 4 suggest that at rapidly lethal concentrations (0.071 mg/l HCN), exercised trout die about 6 h sooner than the non-exercised trout. At the

slowly lethal concentration of 0.062 mg/l HCN the reverse trend occurred when the survival time for the exercised trout was 8 h longer than the non-exercised trout. While these results only show trends because the values in Table 4 are not significantly different, they agree with reports reviewed by Doudoroff (1976) and the findings of Kovacs and Leduc (1982a). These studies found that at low lethal concentrations of cyanide, there is a positive correlation between resistance and metabolism, which is reversed at rapidly lethal concentrations ($>$ 0.100 mg/L). Thus it is felt that in combination with Kovacs and Leduc's (1982a) finding, the trend for a similar response is found in the results of this study. This concentration dependence is best explained by Matida (1960). The physiological and biochemical processes leading to death may be different in short term exposure to high concentrations of a toxicant compared to long term exposure to dilute lethal solutions. It is believed that the exercised trout have higher uptake rates of cyanide than non-exercised trout because of a higher ventilation rate. Increased irrigation of the gills brings in more cyanide. It is postulated that at rapidly lethal concentrations, the exercised trout absorb more cyanide which may reach and deleteriously affect the vital organs before the trout can begin detoxifying the cyanide. At lower lethal levels of cyanide, the exercised trout still take up cyanide faster, but detoxifying mechanisms would have the opportunity to protect the fish (Jones 1964).

The exercised trout may also be more resistant due to increased efficiency or activity of the cytochrome chain as exhibited by fish maintained at higher temperatures (Ekberg 1958, 1962). As well, exercised rainbow trout have an improved oxygen supply and have larger mitochondria (Davison and Goldspink 1977) which may lead to increased mitochondrial activity and to more ATP production and availability. It is believed that only 2/3's of the oxidative system is cyanide sensitive (Jones 1964). Raymond (1984) observed a 60% reduction of the cyctochrome oxidase enzyme for 21 days in 30 g non-exercised rainbow trout exposed to 0.010, 0.020 and 0.030 mg/L HCN. Whether or not this occurs in exercised trout is unknown but should be tested in the future. The cytochrome system in exercised trout may not be inhibited as much and/or that exercised trout may cope better at 1/3 capacity than the non-exercised trout. Fish that can better cope with anoxic stress (goldfish, carp, brown bullheads) are more resistant to cyanide than those requiring high levels of oxygen (Jones 1964, Leduc et al 1973).

Trained (exercised) coho salmon (Oncorhynchus kisutch) (Besner 1980) and Atlantic salmon (Salmo salar) (Wendt and Saunders 1973) can build up larger oxygen debt and can better cope with anaerobic metabolism than untrained salmon which would undoubtedly favor a greater resistance to cyanide poisoning.

Leduc (1984) suggests thyroid activity to be an

important factor in cyanide resistance because it is known that thyroxine increases the efficiency of the respiratory chain (Metzler 1977, Tepperman 1980). It is postulated that higher levels of thyroid hormones would confer fish with a higher resistance to cyanide. Massey and Smith (1968) observed an increased activity of the oxidative enzyme system in brown trout (Salmo trutta) when thyroxine was added to the test aquaria. On the other hand, Higgs and Eales (1971) found brook trout (Salvelinus fontinalis) exercised for 2 weeks contained up to 18% more thyroxine than in non-exercised trout, thus inferring increased oxidative activity. In this study, the exercised trout were acclimated to exercise for 2 weeks prior testing. Hence it is conceivable that the induction of thyroxine could have aided in the protection of these exercised trout.

Season

The results of a preliminary acute toxicity test in the summer, on exercised and non-exercised trout, showed that the test concentrations of cyanide were too low to cause sufficient mortality to determine a 96-h LC50 and that there was no difference between exercised and non-exercised trout. This finding was intriguing at first since the range of cyanide concentrations and water temperatures were simialr to that used by Kovacs and Leduc (1982a) who found the 96-h LC50 for non-exercised rainbow trout to be 0.042 mg/l HCN at 12°C. This preliminary test revealed that this "summer"

trout test population were much more resistant to cyanide than those used by Kovacs and Leduc (1982a) which were tested in December (Kovacs personal comm.).

Exercised trout maintained a fairly uniform and stable response to cyanide from summer to winter while the non-exercised trout experienced a sharp drop in resistance (22%) from summer to winter (Figure 3). To test the hypothesis that non-exercised rainbow trout maintained at an elevated metabolic rate exhibit no seasonal influences on their sensitivity to cyanide toxicity, a lethal bioassay at 18°C was completed in the summer. This summer 96-h LC50 value of 0.076 mg/l HCN was compared with the winter value of 0.068 mg/L HCN obtained by Kovacs and Leduc (1982a) at 18°C (Table 5). As with exercise, this experiment revealed that by increasing the metabolic rate, trout could overcome the influence of season on lethal responses, as the increase in 96-h LC50 from winter to summer was only 8% (same % increase exhibited by the exercised trout) and not significantly higher than the winter value. The median survival times (Figure 5) also illustrate that exercised trout show little change in survival times (sensitivity) from summer to winter. However, the non-exercised trout exhibit a rather important decrease of survival times indicating a marked increase in sensitivity from summer to winter.

Beamish (1964b) has shown that at a constant water temperature, metabolic rates change seasonally, with the consumption of oxygen being less during the winter months.

According to Swift (1964), brown trout are most active in the summer (maximum in June and August) and least active in the winter. It is believed that the difference in metabolic rates between exercised and non-exercised trout is greatest during the winter months and least during the summer. Hence the influence of low level exercise on cyanide toxicity may only be of importance during the winter season.

Another metabolic indicator which has been found to vary from summer to winter is swimming performance. Brett (1964) measured fatigue time of 40 to 50 g sockeye salmon at different times of the year in the swimming chamber of a Blazka respirometer. He observed a 90% decrease in the time to fatigue during the winter (February) when the salmon were tested against a 4.0 lengths/sec current even though they were acclimated to the same test conditions (10°C, 12 h photoperiod) as in summer. This apparent reduction in swimming stamina in winter is likely due to decreased respiratory capacity.

Changes in the thyroid activity may also explain seasonal change of sensitivity to cyanide. White and Henderson (1977) found that thyroid hormones vary seasonally in brook trout with the lowest concentration found during the winter months. Given the role of thyroid hormones in biological oxidation, these seasonal changes could be a determining factor in cyanide toxicity. This aspect remains to be demonstrated and further studies are required to ascertain the role of the thyroid gland in cyanide toxicity.

Seasonal differences in cyanide toxicity with rainbow trout can also be influenced by the genetic background and innate mechanisms of the fish (Bullock 1955). Fish species of the northern temperate zone have evolved to experience seasonal changes in water temperature, nutrient availability, reproductive condition and photoperiod. When these fish are brought into the laboratory from the winter environment, proper acclimation periods must be used as endogenous (genetic) mechanisms can keep these fish from being totally acclimatized to the "summer" laboratory conditions. Previous studies on thermal toxicity clearly show that even though temperature acclimation conditions are the same and the fish are fed similar diets and treated in like manner, resistance to sudden chilling is greater in the winter while resistance to heat is elevated during the summer (Hoar 1959). Observations by Hart (1952) in Houston (1982) and McCauley (1958) show that species from warm climates react differently towards thermal toxicity than the same species reared in colder climates. This was further observed in the offspring of these species. McCauley (1958) obtained eggs from Salvelinus alpinus willughbii (northern England) and S. a. alpinus (France) and reared them together under identical conditions. During thermal testing of this first generation, the English stock showed a lesser heat resistance following acclimation to 10 and 20°C than the French stock. In short, these salmonids exhibited geographic and genetic (innate) distinctions in thermal

tolerance.

Phenol, zinc and ammonia toxicity have been shown to vary seasonally by a factor of 2.5 for the 48-h LC50 values (Ministry of Technology 1968, Brown 1968). Unfortunately these authors do not indicate in which season the toxicants were most toxic. Falk and Dunson (1977) observed a marked seasonal decline (45%) in survival times from early December to late February when brook trout were exposed to lethal pH's of 3.15 to 3.55. They attributed this response to heritable differences. Thus the influence of genotype and innate internal mechanisms may also play a role in controlling seasonal differences in toxicity.

All of these studies demonstrate that there is some sort of an internal biological rhythm that controls the fish's biochemical machinery in yearly cycles, of which resistance to toxic substances such as cyanide are included. The winter non-exercised trout appear to lack total acclimation to the laboratory's "summer" conditions, which may include maintaining a constant response to cyanide. This type of response is also known as acclimatization whereby the organism's previous environmental history is of great importance when testing is completed under laboratory conditions (Hoar 1983) which, in this study, was the running of acute bioassays during the winter. I feel that there is a combination of genetic and environmental factors which may be involved in the seasonal influence on cyanide potency.

Acclimation

Length and conditions of acclimation are very important when testing is conducted throughout the year because of the influence of seasonal cycles. It is generally accepted that a 2-3 week period is adequate for temperature acclimation with fish (Sprague 1973, Houston 1982).

In our study, seasonal changes were observed only in the non-exercised trout after 4 w of acclimation. A longer acclimation period of 10 w enhanced "winter" trout survival to cyanide by 83% (see Table 6). However, survival times were still shorter than for the other spring and summer tests. Even though the acclimation experiment was not carried out until mid-April, it still can be seen that "winter" fish can better acclimatize and begin to react like summer fish sooner than fish acclimated for a shorter period of time. It is also apparent that by increasing metabolic rate (via exercise or temperature) the influence of season can be kept to a minimum, while it is necessary to implement longer acclimation periods for the non-exercised trout to help them overcome the seasonal affect.

Growth Study

The growth study revealed that exercise can have an important modifying influence on sublethal cyanide poisoning. When a fish is kept exercising while being exposed to cyanide, growth stimulation results (Figure 5).

In the absence of cyanide (control groups),

non-exercised trout grew significantly (28%) better than the exercised ones. Besner (1980) found that exercised coho salmon grew much faster (29%) over 120 days, but during the initial 40 days, his exercised salmon grew less than the non-exercised ones (-15%). He also points out that training at 1 length/sec (same speed as in this study) may be too low for a short period of training, but might be more economical on a long-term basis. This may explain why over this 20-day test the exercised control grew less than the non-exercised.

The results of the non-exercised trout exposed to cyanide are comparable to those of Dixon and Leduc (1981) and Kovacs and Leduc (1982b) as there was a slight growth enhancement at 0.005 and 0.010 mg/L HCN but a reduction at 0.020 mg/L HCN. We also observed a growth rebound of 18 and 31 % during the last 10 days amongst the non-exercised trout exposed to 0.005 and 0.020 mg/L HCN. However, the exercised trout, relative to their controls, exhibited the greatest growth enhancement, especially during the last 10 days. Cyanide at 0.005 and 0.010 mg/L HCN enabled the exercised trout to overcome the 28% growth deficit experienced by the exercised control trout. Due to an overall stimulation in growth from day 0, no rebound in growth occured. As with Kovacs and Leduc (1982b), it appears as if the exercised trout (at a high metabolism) have a higher wet weight growth threshold concentration (Figure 5) than the non-exercised trout. At 18°C they found that trout had a growth threshold of 0.030 mg/L HCN, compared to 0.015 mg/L HCN for trout

tested at 12°C. From this study the wet weight growth threshold concentration can be estimated as greater than 0.020 mg/L HCN for the exercised trout and between 0.010 and 0.020 mg/L HCN for the non-exercised ones.

Using the same annular exercise tanks, McCracken and Leduc (1980) found cyanide toxicity to be size related. They exposed rainbow trout to 0.015 mg/L HCN and found small fish (6 - 11 g) grew 22 - 29 % faster than their controls while the larger trout (16 - 27 g) exhibited a 20% reduction in growth. This 22 - 29 % value is very similar to the 28% growth stimulation observed in this study at 0.010 mg/L HCN with trout of the same size. This response may be attributed to the higher metabolic rate of the smaller fish than in the larger ones (Beamish 1964a). Also, juvenile chinook salmon (Oncorhynchus tshawytscha) exposed to 0.010 mg/L HCN for several weeks in an artificial stream (24 cm/sec current), grew better than the controls (Negilski 1973). Work by McCracken and Leduc (1980) also found that the size of the diet modifies growth rates of rainbow trout exposed to sublethal levels of cyanide. As the size of the ration was increased from 0% to 2% body wt/day, the detrimental effect on growth was reduced to such an extent that cyanide exposed trout grew as well or better than controls. Since the trout in this experiment were fed a ration of 2.5% body wt/day, it is unlikely that a stress on energy caused by low levels of cyanide had any significant effect. Since glycogen levels remain fairly constant

throughout exposure to cyanide, it would appear that cyanide has no detrimental effect on stored energy and that energy uptake via the 2.5% ration may provide sufficient energy for the exposed trout. Leduc (1966b) noted that at low concentrations, cyanide promoted higher food consumption, food conversion efficiency and growth. This coupled with the high diet ration could help explain the growth results in the cyanide exposed trout.

This enhancement in growth is not an uncommon response in toxicology, and has become known as hormesis (Stebbing 1982). Leduc (1984) postulates that a rise in thiocyanate levels (through detoxification) may play an important role in the growth of cyanide-exposed fish. Firstly, at low cyanide concentrations (0.005 mg/L HCN), thiocyanate levels can inhibit the function of the thyroid gland. The reduction in thyroid hormones provokes the stimulation of thyrotropin (TSH) secretion from the pituitary gland which may interfere with gonadotropin production (Sing et al. 1977). Eales and Shostak (1983) observed reduced levels of T4 (thyroxine) while T3 (tri-iodo-thyroxine) remained constant or increased in rainbow trout following injection of KSCN: thus the T4:T3 ratios are altered in favor of T3. Since T3 is more biologically active, the overall potency of the secreted thyroid hormone would be greater and, in conjunction with depressed gonadotropin, could cause increased growth rates soon after exposure to low concentrations of cyanide. When catfish (Heteropneustes

fossilis) were exposed to KSCN, Sing et al.(1977) observed depressed thyroid activity, accelerated thyrotropin synthesis and lessened gonadotropin concentrations which resulted in retardation of gonadal activity. The "extra" energy not used for gonadal growth could be directed towards somatic growth. It is also noted that reduction of reproductive potential has always been observed at the cyanide concentrations that augment growth. Cheng (1978) observed the latter, when newly fertilized American flagfish (Jordanella floridae) eggs were exposed to 0.065, 0.075 and 0.087 mg/L HCN from fertilization to hatching, but returned to clean water afterwards. These newly hatched fish displayed enhanced growth but delayed sexual maturity. This aspect of greater growth at the expense of reproductive maturation has been well documented and is of major concern in aquaculture production. It is known that the size distribution of Atlantic salmon parr exhibits bimodality during their first year of rearing (Thorpe 1977). Sexually mature males (precocious parr) are found predominately in the lower mode (smaller fish) while large immature parr are found in the upper mode (Bailey et al 1980; Saunders et al 1982). The bimodal distribution of sizes suggests some sort of physio-biochemical alteration in the fish's growth patterns. Either a fish will be sexually mature at the expense of reduced growth or show better somatic growth at the expense of reproductive viability.

Another explanation of this growth stimulation

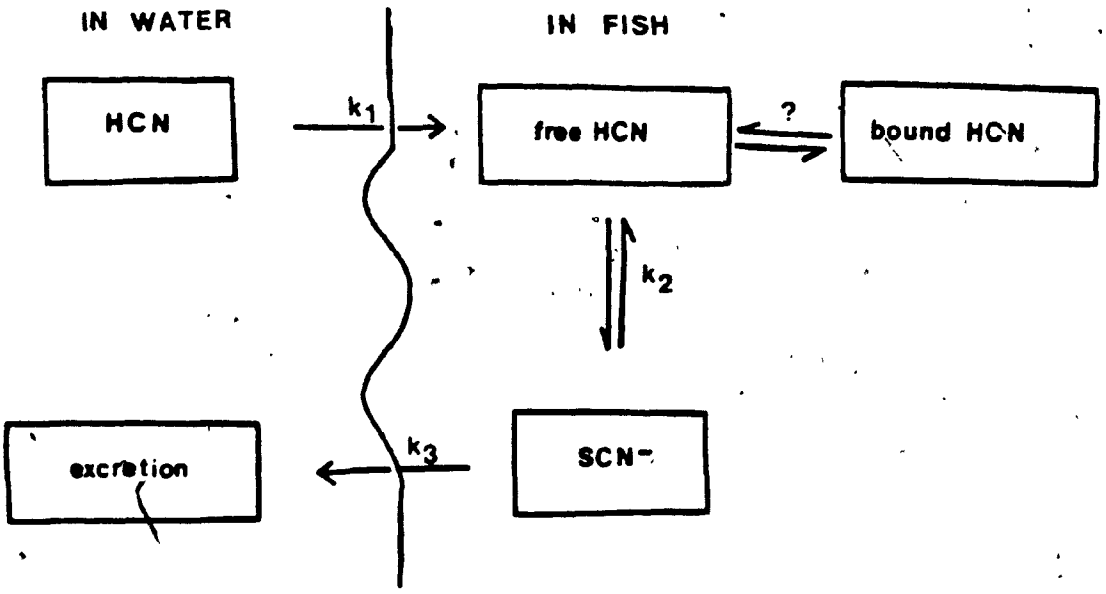
phenomenon has to do with the thyroid gland as well. As previously observed by Raymond (1984), this study reveals that exposure to cyanide produces an accumulation of thiocyanate which may inhibit iodide transport into the thyroid gland, reduce levels of thyroxine and thus depress growth. Iodide builds up in the blood plasma because its uptake is blocked by thiocyanate. The reduction in thyroid hormones causes a feedback reaction to the pituitary, thereby elevating circulating thyrotropin levels (Tepperman 1980). After a certain period of time, iodide accumulates in the plasma until it becomes high enough to overcome the thiocyanate inhibition. This would also occur when thiocyanate levels are leveling or dropping at 10 to 15 days after cyanide exposure. As a result of this, a surge of iodide will enter the thyroid and production of hormone could resume but at a much higher rate than a gland that has not been deprived beforehand. This elevation in thyroid hormones could increase growth rates in the cyanide exposed trout since marked increases in growth of fish fed T3 or T4 have been observed (Higgs et al 1976, Higgs et al 1979). Since the exercised trout exhibited growth stimulation within the first 10 days of cyanide exposure, this feedback reaction may take place over a shorter period of time than in the non-exercised trout. In addition Massey and Smith (1968) relate increased specific activity of oxidative enzymes and increased growth rates to high levels of thyroxine.

The nature and exact explanation of this growth response in the exercised trout is not fully understood. A more complete, comprehensive energetics study is needed to better answer this question of altered but "benefical" growth response in exercised trout.

Thiocyanate Bioaccumulation

The detoxification of cyanide into thiocyanate is mediated through the enzyme rhodanese which has been found in fish liver (Sido and Koj 1972). Raymond (1984) has shown that thiocyanate bioaccumulates in the blood plasma during exposure of non-exercised rainbow trout to sublethal concentrations of cyanide in the water. This study by Raymond (1984) was the only one found on thiocyanate bioaccumulation in fish, while other works dealt with mammals or birds (Bourdoux et al 1979; Barrett et al 1979 and Davis 1981).

The bioaccumulation of thiocyanate in the blood plasma of the trout may be considered in the following model:



First, thiocyanate levels will depend on the uptake of cyanide from the water (k1) and then on its conversion into thiocyanate by rhodanese (k2). The reverse reaction is unclear but the equilibrium is largely towards thiocyanate production (Westley 1981). Thiocyanate is a pseudophalogen and it will therefore be reabsorbed by the kidney tubules but is never-the-less excreted (Davis 1981) (k3) when it exceeds a certain level. So when monitoring plasma thiocyanate levels, these three rate constants k1, k2, and k3 must be considered since they can be influenced by metabolic rate (exercise).

As in Raymond's (1984) work, this study found thiocyanate to bioaccumulate within 48 hours with the levels increasing until Day 7. Beyond Day 7, thiocyanate accumulation tends to level off which could be the result of increased excretion (k3). In Experiment 2, the exercised trout exhibit slighlty different patterns in accumulation which can be attributed to exercise. However the different levels of thiocyanate accumulation between experiments 1 and 2 could be the result of using different size trout and/or to season. Since the size of the trout were not controlled from Experiment 1 (summer) to Experiment 2 (winter), it is hard to make a direct correlation between thiocyanate levels and season. However, within each experiment size is the same. The results from the thiocyanate bioassays can be used to help explain the findings of the acute toxicity tests. It can be seen (Figure 6) that during the summer

there are no differences in thiocyanate bioaccumulation between exercised and non-exercised trout. This correlates well with the summer acute toxicity results when 96-h LC50 values for exercised and non-exercise trout were the same. These similar LC50 values could reflect similar responses in detoxification rates. However, during Experiment 2 (winter) the exercised trout accumulate thiocyanate faster until Day 7, after which levels drop off on Day 15. Hence, the exercised trout either detoxify cyanide more rapidly than the non-exercised trout (k2) or they have a faster uptake (k1) of cyanide than the non-exercised trout. In either case it is evident that the exercised trout react differently to cyanide poisoning during the winter than do the non-exercised trout which suggests a greater resistance to cyanide in the winter. Overall it can be seen that cyanide exposure is related to thiocyanate accumulation and the detoxification mechanism starts within 48 hours or earlier. Monitoring rhodanese activity would also expand the knowledge of the cyanide detoxification model.

As mentioned earlier, the growth stimulation responses in both exercised and non-exercised trout can be linked to the variation in thiocyanate levels (rising and leveling off or falling) and its effects on the thyroid gland.

SUMMARY

This study reveals two major findings. Firstly, exercise increases resistance of rainbow trout to cyanide and secondly, there is a seasonal variation of sensitivity to cyanide with resistance being lowered during the winter. This seasonal response is most likely due to circannual cycles of biochemical pathways within the fish which may make the fish more susceptible to cyanide in the winter. By increasing the metabolic rate through exercise or higher temperature, this seasonal phenomenon can be kept to a minimum or even eliminated. This altered response exhibited by the exercised trout is most likely due to a higher metabolic rate, and a better ability to withstand anoxic stress such created by cyanide.

In the absence of cyanide, exercise lowered growth rates over 20 days (-28%) as compared to non-exercised trout. However, when both exercised and non-exercised trout were exposed to sublethal concentrations of cyanide, exercised trout exhibited growth stimulation which enabled them to overcome this deficit observed in the controls as the growth rates of these trout were similar to each other. The glycogen results indicated that there does not appear to be much of an energy strain on the trout. This may account for the lack of growth depression since the low cyanide test concentrations used in this study do not appear to have much of an effect on energy reserves. The slight growth

increases in non-exercised trout and the larger increases of growth in exercised trout could also be related to thyroid activity and the influence of thiocyanate on T3 and T4 production.

During the winter the exercised trout accumulate thiocyanate faster and in greater amounts than the non-exercised trout which correlates well with the winter acute toxicity tests. Their detoxification and excretion appear to be better than the non-exercised trout during the winter period. A more controlled experiment monitoring cyanide uptake levels and thiocyanate excretion rates are needed to better understand the complete picture of cyanide toxicity. Monitoring rhodanese activity would also expand the knowledge of the cyanide detoxification model. These seasonal differences in cyanide poisoning may be the result of seasonal cycles in respiration rates, respiration capacity, respiratory enzymes (cytochrome oxidase) or thyroid hormones. More research needs to be done in this area.

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Appendix 1. Median survival time (MST) and confidence intervals of exercised and non-exercised rainbow trout exposed to various concentrations of cyanide at 12°C during different seasons throughout the year.

Season / Treatment	HCN (mg/L)	MST (hours)	95% confidence interval
Late Summer			
Exercised	0.000	*	--
	0.045	*	--
	0.055	*	--
	0.062	50	45 - 55
	0.067	32	26 - 40
	0.071	20	16 - 25
Non-Exercised	0.000	*	--
	0.044	*	--
	0.052	*	--
	0.063	42	36 - 50
	0.068	36	29 - 44
	0.070	26	22 - 30
Winter			
Exercised	0.000	*	--
	0.038	*	--
	0.044	*	--
	0.053	79	--
	0.073	20	18 - 23
Non-Exercised	0.000	*	--
	0.033	*	--
	0.040	105	72 - 153
	0.044	66	57 - 76
	0.052	29	23 - 35
	0.062	27	24 - 30
Spring			
Exercised	0.000	*	--
	0.038	*	--
	0.042	*	--
	0.049	*	--
	0.058	96	--
	0.066	40	30 - 53
Non-Exercised	0.000	*	--
	0.035	*	--
	0.040	*	--
	0.045	*	--
	0.052	*	--
	0.068	45	36 - 57

This symbol * indicates MST > 96h

Appendix 2. Median survial times (MST) and confidence intervals of non-exercised rainbow trout exposed to various concentrations of cyanide at diffferent temperatures during the summer.

Temperature	HCN (mg/L)	MST (hours)	95% confidence interval
12°C	0.000	*	---
	0.045	*	---
	0.050	*	---
	0.055	*	---
	0.062	85	---
	0.070	32	27 - 38
18°C	0.000	*	---
	0.056	*	---
	0.065	*	---
	0.075	*	---
	0.085	28.5	22 - 36
	0.100	15	11 - 19

This symbol * indicates MST > 96h