THE INFLUENCE OF TEMPERATURE ON THE ACUTE AND CHRONIC TOXICITY OF CADMIUM TO RAINBOW TROUT

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ABSTRACT

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The Influence of Temperature on the Acute and Chronic Toxicity of Cadmium to Rainbow Trout

Acute toxicity of cadmium to rainbow trout was determined by continuous flow bioassay after acclimation to the test temperatures of 6, 12 and 18°C. Median survival time and ten day lethal threshold concentrations were inversely related to temperature, and accumulation of cadmium in gill, liver, kidney and whole fish was more rapid at 18° than at 6°C.

Analysis of variance of the per cent wet weight gains following twenty days of sublethal exposure to cadmium did not reveal a significant difference among treatments. However, mean per cent wet weight gains appear to be reduced at the higher cadmium concentrations in comparison to the controls. Calcium concentrations in the blood were elevated at cadmium concentrations of 4.2 ug Cd/L and above, and the increases were more pronounced with increasing temperature. Although significant increases in magnesium concentrations occurred, they were not as pronounced as the calcium elevations. Sodium and potassium concentrations did not show marked changes. Cadmium residues in gill, liver, kidney and whole fish show a trend toward increased accumulation rate with increasing temperature.

A third experiment was carried out to determine the effect of acclimation on the acute response of the trout to 0.3 mg Cd/L. Survival times of 12°-acclimated fish that were exposed to cadmium following abrupt transfer to 6, 12 and 18°C, were temperature dependent, whereas the trout that were acclimated to test temperature showed a marked deviation from temperature dependence. Fish acclimated to 18° and exposed to cadmium at 18° survive twice as long as 12°-acclimated trout exposed at 18°C following abrupt transfer.

Calcium concentrations in the plasma of trout that were exposed to 0.3 mg Cd/L, declined steadily during the exposure period. The time at which they had declined to 3.0 meq/L was significantly correlated with survival time at five combinations of acclimation and exposure temperature. The symptoms of acute cadmium poisoning are consistent with known effects of hypocalcemia and suggest that extreme hypocalcemia is the cause of mortality.

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Introduction

Cadmium has become recognized in recent years as a significant and highly toxic environmental contaminant (Friberg et al. 1971, Nordberg 1974, Fassett 1975). Although the toxicity of cadmium has been known for some time, the reports of chronic cadmium poisoning in Japan have stimulated concern with cadmium toxicology.

Aquatic organisms are particularly sensitive to the toxic effects of cadmium (EIFAC 1978, EPA 1976) and may accumulate the metal to high concentrations in their tissues in areas of cadmium pollution (Murphy et al. 1978). Little is known of the toxic action of cadmium at either the acute or sublethal level and the effect of temperature on toxic response and accumulation rates in fish. This study is an attempt to clarify some of these relationships in the rainbow trout.

Cadmium-uses and occurrence

Cadmium is used in a wide variety of consumer goods and its yearly production in the United States has increased from 100,000 lbs. in 1910 to 31,000,000 lbs. in 1968 (Fassett 1975), probably due to the demand for its corrosion resistant properties. Sources of cadmium contamination in air and water are mainly lead, copper and zinc mines, as well as smelting operations which extract cadmium from these ores. Contamination may result from industries which use cadmium, particularly electroplating, paint, plastic and chemical works. Use of cadmium in nickel-cadmium batteries, in the production of alloys and solders and as fungicides, contribute to a major portion of the consumption (Page and Bingham 1973).

Canada, which is one of the world's largest cadmium producers, yielded 2.7 million pounds in 1975 (Ministry of Supply and Services,

Canada, 1977). The largest mine production occurs at the Kidd Creek Mines at Timmins, Ontario. Other important sources include the Geco Mines at Manitouwadge, Ontario, Cominco Ltd. at Trail, B.C. and Hudson Bay Mining and Smelting in Flin Flon, Manitoba. Noranda Mines in Ontario, Quebec and New Brunswick, Pine Point Mines in the North-west Territories, and Anvil Mining Corporation in the Yukon Territory also produce significant amounts. Metallic cadmium is recovered as a byproduct from electrolytic zinc plants in Trail, B.C., Flin Flon, Manitoba, Timmins, Ontario and Valleyfield, Quebec (Ministry of Supply and Services, Canada, 1977).

Page and Bingham (1973) compiled data on cadmium concentrations in over 2,000 samples of water from different areas in the United States and report that natural waters usually contain less than 1 ug/L of cadmium and that the incidence of cadmium concentrations in excess of 15 ug/L is extremely rare. Kopp and Kroner (1967) reported elevated levels of cadmium in less than 3 per cent of 1,577 water samples with a mean of slightly less than 10 ug/L, which is the recommended safe limit for domestic water supplies (EPA, 1976). Kopp and Kroner (1967) reported levels in excess of 10 µg/L in 6 of the 1,577 samples, one of which was 120 µg/L in the Cuyahoga River which flows into Lake Erie.

Water in regions close to mining and smelting operations as well as industries producing or using cadmium products may contain high concentrations of cadmium. Studies of chronic cadmium poisoning ("itai-itai" disease) in a cadmium contaminated area of Japan revealed high concentrations of cadmium in river water, rice and fish downstream from a lead, zinc and cadmium mine. Three samples from the drainage of the mine contained 5-60 µg/L

of cadmium (pH 7-8), and one contained 4,000 µg/L (pH 2.8). Cadmium concentrations of 363-382 ppm were found in suspended matter near the mine (Friberg et al. 1971, p.125). Stokes et al. (1973) report cadmium concentrations of between 3 and 20 µg/L in six of nine lakes near the Sudbury area, as well as high concentrations of copper, nickel and zinc. Van Loon and Beamish (1977) report 50 µg Cd/L in Ross Lake which receives effluent from the Flin Flon, Manitoba mining and smelting complex. John et al. (1975) report extremely high concentrations of cadmium and other heavy metals in the soil near the copper, lead and zinc smelting operations at Trail, B.C. The Columbia River below Trail shows elevated zinc concentrations with a median of 40 µg/L (Environment Canada, Water Quality Data for B.C., 1974). Although cadmium levels were not reported, they may be elevated as well due to groundwater leaching of the high concentrations in the soil and the reception of effluent from the smelting complex.

Mink and Williams (1970, cited in Page and Bingham 1973), reported a cadmium concentration of 450 µg/L in the Coeur d'Alene River basin of Idaho near a mining and smelting complex. Although cadmium concentrations in Canadian waters have received little attention, they may pose or contribute to a significant threat to fish in areas of cadmium production and use.

Toxic effects of cadmium

The toxic effects of cadmium on humans and mammals have been extensively reviewed (Friberg et al. 1971, Flick et al. 1971, Nordberg 1974, Fassett 1975). However, little is known of cadmium toxicity to fish or of levels which might pose serious threats to fish. Ball (1967), using rainbow

trout, found that the seven-day LC50 at 12°C was approximately 10 ug Cd/L in hard water. Kumada et al. (1973) report a similar ten-day LC50 of 7 ug Cd/L for three gram rainbow trout at 16.5°C in water of unspecified hardness. Pickering and Gast (1972), using fathead minnows, determined a seven day LC50 of 7.2 mg Cd/L in water of moderate hardness. Pickering and Gast's results in comparison to those of Ball and Kumada indicate that different species of fish vary a great deal in their susceptibility to cadmium, and that salmonids may be extremely sensitive to its toxic effects. Benoit et al. (1976) have shown the brook trout (Salvelinus fontinalis) to be extremely sensitive to the deleterious effects of cadmium as well. They established a maximum acceptable toxicant concentration (MATC) of 1.7-3.4 ug Cd/L on the basis of no reduction in survival or reproduction of three generations of brook trout.

Eaton et al. (1978) exposed embryos, larvae and juveniles of seven fish species to cadmium for periods of 27 to 126 days and found that the highest concentration which did not reduce the standing crop ranged between 1.1 and 4.4 ug Cd/L in all cases. They suggest that an effective measure of chronic cadmium toxicity can be provided by 60 days exposure of the larvae, which in their case was the most sensitive stage. Pickering and Gast (1972) found embryos to be the most sensitive life stage of the fathead minnow and Spehar (1976) found impaired reproduction of the flagfish to be the most sensitive response. The use of one life stage to determine a safe level of cadmium does not appear to provide an accurate reflection of the toxic hazard of cadmium.

Bioassays to determine maximum acceptable toxicant concentrations in terms of survival or growth provide little understanding of the

subtle biochemical effects which may occur as a result of exposure to cadmium. Knowledge of these effects might allow more adequate predictions of the threat which cadmium could pose to a population of fish in the natural environment.

Bengtsson et al. (1975) report that 31 of 101 minnows (Phoxinus phoxinus), surviving chronic cadmium exposure for seventy days, developed lesions of the spinal column. Skeletal abnormalities were observed at concentrations as low as 7.5 ug Cd/L. Although mature fish were used in this study, the effect could be more profound in immature stages with a rapidly developing skeletal system. Eaton (1974) observed skeletal deformities of larval bluegills exposed to cadmium. Although fish with skeletal damage might survive in a sheltered laboratory milieu, it is unlikely that they could survive the stresses of the natural environment for long periods of time.

Chronic studies indicate that cadmium is extremely toxic and that small elevations above natural levels can cause serious damage to fish. Bengtsson et al. (1975) point out that levels of 5-10 ug Cd/L have been reported in the Baltic Sea and suggest that contamination may already have caused chronic vertebral damage to fish in that area.

The extensive evidence of vertebral damage in fish and the osteomalacia of patients chronically exposed to cadmium may be related symptoms. Friberg et al. (1971) report consistently low calcium levels in the serum of "itai-itai" patients and a disturbance in calcium metabolism would be expected in patients that suffered symptoms of osteomalacia.

Bengtsson et al. (1975) report hyperactivity as being a symptom

of cadmium exposure and observations of hyperactivity in fish are common (Pickering and Gast 1972, Benoit et al. 1976, Eaton 1974, Cearley and Coleman 1974, Pascoe and Matthey 1977). This symptom of cadmium toxicity in fish may be consistent with a disturbance in calcium metabolism. Calcium regulation is important to proper nerve and muscle function and a disturbance could produce symptoms of hyperactivity and uncontrolled muscle activity.

In view of the evidence relating disturbances in calcium regulation with the toxic action of cadmium, I decided to investigate calcium concentrations in the blood as a potentially sensitive indicator of acute and chronic cadmium toxicity.

Temperature and toxicity

Temperature is an important controlling factor of metabolism in poikilotherms and can be expected to have a profound influence on the toxic response to a pollutant. In order to predict the toxicity of certain heavy metals in a temperate environment, relative toxicity at different temperatures must be established. Although temperature has been recognized as an important variable affecting toxicity, and attempts are generally made to control temperatures in bioassays, few attempts have been made to establish toxicity at different temperatures.

It is often assumed that toxicity will increase with temperature because the metabolism of a fish increases with temperature. Hodson and Sprague (1975) have shown the converse relationship to be true with respect to zinc toxicity to Atlantic salmon (Salmo salar). Although survival time was longer at low temperatures, the threshold

LC50 was also lower, indicating that the fish were less tolerant at low temperatures. Such anomalies point out the need for more investigation of the physiology of fish in relation to temperature and the need for more careful evaluation of the fish's response to heavy metals at different temperatures.

Fish acclimated to a certain temperature show a logarithmic increase of metabolic rate as the temperature is raised rapidly (Wieser 1973, Peterson and Anderson 1969), whereas fish that are acclimated to different temperatures may have different metabolic rates at the same test temperature. The experiments of Peterson and Anderson (1969) show that 6°C-acclimated Atlantic salmon (Salmo salar) have a metabolic rate that is 56% higher than that of 18°-acclimated salmon when both are exposed to 6°C. These experiments indicate seasonal thermal adaptation which allows the fish to compensate for the high metabolic activity coupled with low oxygen availability that is dictated by the high kinetic energy of molecules at high temperatures. The relatively higher cold adaptation metabolism may be a response which allows the fish to maintain sufficient activity for growth and survival at the slow biochemical reaction rates characteristic of low temperatures during winter conditions.

Ectothermic organisms often undergo extensive biochemical reorganization during metabolic rate compensation and thermal adaptation. For example, cold-acclimated fish may use the pentose-phosphate pathway of glucose catabolism to a greater extent (Hochachka 1968). There is also extensive evidence of qualitative changes in enzymes with changes in acclimation temperature (Hochachka and Somero 1973). One example of an enzyme which shows a qualitative change with temperature is acetylcholinesterase in

rainbow trout. Baldwin (1971) found two different forms of the enzyme in rainbow trout acclimated to 2° and 17°C; at 12°C both forms existed simultaneously.

These extensive biochemical changes in fish acclimated to different temperatures may have varying susceptibility to a toxicant depending on mode of action. The response of a fish may be independent of the dictates of greater kinetic energy at higher temperatures that might increase the interaction between a toxicant and its site of action. Since little is known of the biochemistry and physiology of temperature adaptation in fish and in many cases modes of action of heavy metals are poorly understood, it is important to evaluate the toxic effect of a heavy metal over a range of temperatures to which a fish might be exposed in its natural environment. This information would give a more complete assessment of the hazard that a pollutant represents to a population of fish during different seasons.

Objectives

The absence of information on the acute toxicity of cadmium at different temperatures made it prerequisite to determine median survival time and approximate incipient lethal level. In the first experiment, rainbow trout acclimated to a cold (6°C), an intermediate (12°C) and a warm temperature (18°C), were exposed to a range of cadmium concentrations at the temperature of acclimation. Accumulation of cadmium in gill, liver , kidney and whole fish at mortality was intended to provide information that might correlate with mortality. These tissue residues would also provide relative accumulation rates under the three temperature regimes.

A second twenty-day experiment was carried out at the three temperatures to determine any changes in the divalent cation concentrations of the blood that might be due to cadmium exposure. The sublethal experiment was also designed to determine reductions in growth that might have resulted from interference with respiration or energy-requiring processes. Per cent dry weight determinations were intended as a measure of osmoregulatory disturbances that might result from impairment of gill or kidney function. Analysis of cadmium residues in tissues were performed to determine relative accumulation rates at the different temperatures and whole fish residues were examined to determine whether the fish could accumulate low concentrations of cadmium to levels that might pose a health hazard if the fish were consumed.

In view of the extensive biochemical and physiological changes which take place in fish during acclimation to a specific temperature, a third experiment was designed to determine whether the acute response of non-acclimated trout differed from that of trout acclimated to the temperature of exposure. Fifty per cent survival time was determined and changes in the divalent cations and plasma osmolality were determined at intervals during the exposure period in an attempt to relate changes in ion concentrations with mortality.

Material

Test organism

Juvenile rainbow trout (Salmo gairdneri Richardson) were obtained from La Pisciculture Mont Sutton at Mont Sutton, Quebec. The fish were transported to Concordia University in plastic bags inflated with oxygen. Temperature change during transport was minimal and mortality was less than one per cent.

The trout were placed in a 300 litre holding tank on arrival at the laboratory. Oxygen concentrations were maintained by constant aeration. Flow of water was six litres per minute. The trout were fed a mixture of no. 3 and no. 4 trout chow (Ewos) at a ration of two per cent of body weight per day. The holding tank was siphoned daily before feeding and scrubbed once per week. The fish were allowed to acclimate to laboratory conditions at 12°C for two weeks prior to temperature segregation for the first experiment.

Water characteristics

City of Montreal water, dechlorinated by charcoal filtration, was delivered to test tanks through PVC piping. The laboratory receives water maintained by the physical plant of the building at constant temperatures of 11° and 27°C. The cold water (6°C) was obtained by cooling 11° water with a chiller (Dunham Bush of Canada Ltd. Model PC5 HO). A thermopanel (Series 420 Hydroguard. Powers Regulator Co., Skokie, Ill.) maintained a mixture of 11° and 27° water at the warm temperature (18°C). The intermediate temperature (12°C) was obtained in the test tanks after slight warming of the supply water by the room temperature. Problems of supersaturation arising from the heating of water under pressure were avoided

by means of a mechanical degassing unit.

Dissolved oxygen, alkalinity and hardness were determined according to the American Public Health Association (1971) methods. Oxygen in supply water was always above 90% saturation, hardness ranged from 125-133 mg/L as $CaCO_3$ and alkalinity was between 88 and 92 mg/L as $CaCO_3$. pH was 7.7-7.9 throughout the experimental period. Water characteristics and fish sizes during the three experiments are given in Table 1.

Exposure apparatus

The experimental apparatus consisted of 24 rectangular polyethylene tanks each with a central standpipe drain which maintained the volume of water at ninety litres. Figure 1 is a diagram of one experimental tank. Three head boxes supplied a constant pressure of water at the three experimental temperatures. Each head box supplied eight tanks with water at the appropriate temperature and the flow rate into each tank was regulated at 750 ml/minute by flow meters (Manostat Corp., New York, N.Y.). This rate gave a 95% replacement time of 6 hours and provided adequate water to sustain oxygen requirements and remove wastes (see Sprague 1973).

Cadmium solution was delivered from eighteen Mariotte bottles (see Leduc 1966) through capillary tubing and mixed in funnels with the diluent water before entering the test tanks. The height of the capillary tubing was adjusted to give a constant toxicant flow rate of 1.5 ml/minute. The toxicant, $CdCl_2 \cdot 2\frac{1}{2}H_2O$, was reagent grade material assayed at 79.5-81.0% as $CdCl_2$. The cadmium was dissolved in eighteen litres of dechlorinated water acidified to pH 6 with sulphuric acid. Concentrations in Mariotte bottles were calculated to be 500 times the exposure concentration as Cd^{2+}.

Table 1: Fish sizes and water characteristics during the three experimental periods

(* 95% confidence limits)

	Temp (range °C)	D.O. (% sat.)	Hardness	pH	Alkalinity	Fish wt. (g.)	Fish length (cms)
Expt.1 Dec. 1977	6 ± .5	> 90%	128 mg/l ($as\ CaCO_3$)	7.8	90 mg/l ($as\ CaCO_3$)	9.34 ± .55*	9.4 ± .2*
	12 ± .5	> 90%				9.78 ± .64	9.5 ± .2
	18 ± .5	> 90%				8.55 ± .48	9.1 ± .2
Expt.2 Feb. 1978	6 ± .5	> 90%	133 mg/l	7.9	92 mg/l	13.15 ± .67	10.6 ± .2
	12 ± .5	> 90%				15.24 ± .76	11.1 ± .2
	18 ± .5	> 80%				14.52 ± .79	10.8 ± .2
Expt. 3 June 1978	6 ± .5	> 90%	124 mg/l	7.8	88 mg/l	19.90 ± 1.08	12.5 ± .2
	12 ± .5	> 90%				20.01 ± 1.20	12.6 ± .2
	18 ± .5	> 80%				19.52 ± 1.02	12.6 ± .2
	6 (12°C-acclimated)					19.02 ± 1.06	12.4 ± .2
	18 (12°C-acclimated)					21.49 ± 1.55	12.7 ± .3

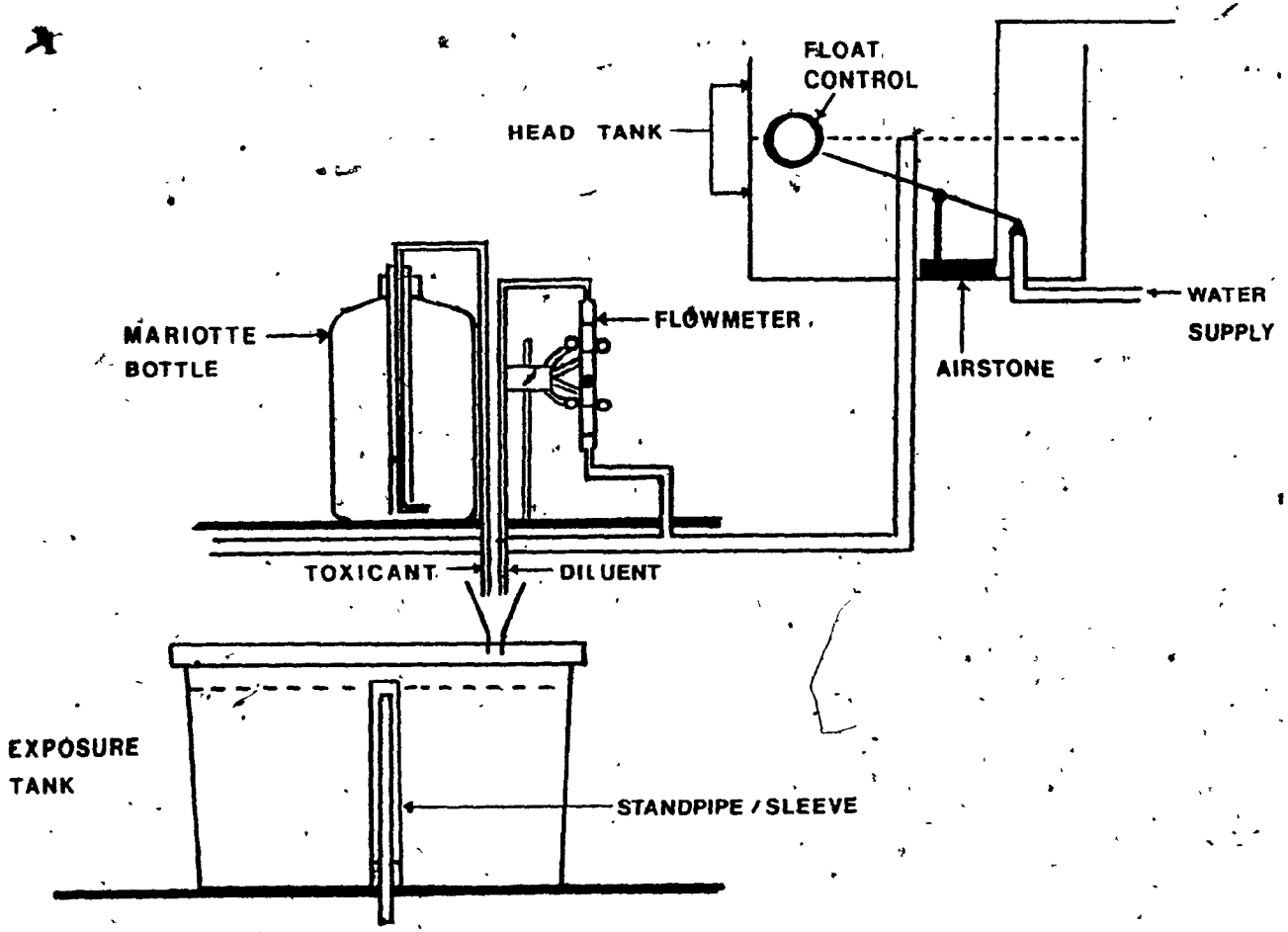


Figure 1: Diagram of the continuous flow apparatus including one of twenty-four exposure tanks and one of three head tanks. Mariotte bottles delivered cadmium solution to the test tanks and the flow of diluent water was regulated by a flow meter.

Illumination was provided by fluorescent lighting and the photoperiod was maintained at 12 hours light/12 hours dark throughout all experiments. All tanks were wrapped with dark plastic to minimize disturbance except the eight 6° tanks which were encased with styrofoam insulation. The experimental tanks were covered with screens and sheets of dark plastic.

Experimental Methods

Analysis of cadmium concentrations

Daily twenty millilitre samples of the exposure water were taken during the experimental period and acidified with a drop of sulphuric acid. Three composite samples of each concentration were assayed by atomic absorption spectrophotometry (Perkin-Elmer model 503). Concentrations of 30 µg/l and less were analyzed by the graphite furnace technique. All concentrations of cadmium in the water refer to mean assayed values of total cadmium during the exposure period. Concentrations of cadmium in City of Montreal water were less than 1 µg/l with a mean of 0.5 µg/l.

Acclimation procedure

Temperature was adjusted from an initial 12°C at a rate of one degree per day and the fish were maintained at the proposed test temperatures for at least two weeks before any experiment was begun. Peterson and Anderson (1969) found that Atlantic salmon (Salmo salar) required two weeks to adjust their metabolism after a temperature change of 6-18 or 18-6°C. Two weeks acclimation was therefore deemed adequate for full acclimation following the less extreme (six degrees) temperature changes in this experiment.

During the acclimation period, the fish were fed trout chow at a

ration of two per cent of body weight per day and the tanks were siphoned daily before feeding. Water flow was adjusted to provide more than three litres per gram of fish per day, which is recommended by Sprague (1969) as an adequate flow rate. Mortality during all acclimation periods was minimal and all fish used for the experiments were apparently healthy and feeding well.

Analysis of cadmium residues in fish tissues

Gill, liver and kidney were dissected from fish that had been exposed to cadmium and dried to constant weight in an oven at 80°C. Extraction of cadmium was performed according to the method of Leonard (1971) . Benoit et al. (1976) report 98% recovery of cadmium from spiked brook trout tissues using this method. Whole fish were dried in an oven to constant weight and composite samples of five fish were homogenized in a blender; 0.2-0.25 g of the homogenate were used for analysis of cadmium residues.

Cadmium residues were determined by flame atomic absorption and by graphite furnace for levels below the limit of sensitivity of the flame apparatus.

Blood ion analysis

Blood was collected by severing the caudal area to obtain a composite sample of blood from all fish exposed to a particular concentration and temperature. Blood samples from the second experiment were allowed to clot and were then centrifuged. The blood cells were later resuspended by vortexing and a 0.1 ml sample of blood cells and serum was drawn by serological pipette. The sample was diluted with 1.9 mls of a solution containing 0.1% lanthanum and 5% trichloroacetic acid (Trudeau and Freier 1967). Lanthanum was added to prevent interference from phosphate

and sulphate and the TCA was added to disrupt the blood cell membranes, to separate calcium bound to proteins and to prevent interference of proteins with the atomic absorption analysis (see Berman 1975). Ion concentrations reported are representative of serum and blood cells. Following the precipitation of proteins by centrifugation, a 1 ml sample of the supernatant was diluted to 5 mls with 0.1 % lanthanum solution. Three replicates treated in this manner were analyzed for calcium and magnesium by flame atomic absorption using appropriate standards. Further dilutions of 1:20 were analyzed for sodium and potassium.

After the third experiment blood from five to ten fish was collected into heparinized tubes according to temperature and time of exposure to cadmium. Triplicate 50 µl samples of plasma were diluted to 5.0 mls with 0.1% lanthanum solution. Calcium and magnesium concentrations were determined by flame atomic absorption.

Three 0.2 ml replicates of each plasma sample were used to determine osmolality by freezing point depression using a Technicon Auto Analyzer.

Experimental Design

The first experiment was intended to determine incipient lethal concentrations at three temperatures (6, 12 and 18°C) by measuring median survival time (ET 50) of rainbow trout exposed to a range of cadmium concentrations.

After acclimation to test temperature, seventy fish were divided randomly among seven tanks at each of the three test temperatures. Following a 48 hour period of starvation and acclimation to test chambers a quantity of cadmium solution was added to the water to attain the desired concentration. Flow rates were then adjusted to give the required toxicant flow and dilution factor.

The fish were exposed to six concentrations at equal logarithmic intervals from 0.032 to 10 mg Cd/L at 6 and 12°C. At 18°C, the trout were exposed to six nominal concentrations at logarithmic intervals from 0.01 to 3.2 mg Cd/L.

Observations of mortality were recorded following a logarithmic series suggested by Sprague (1973). Mortality was regarded as lack of response to prodding and lack of opercular movement. Fish were removed as soon as mortality was observed, rinsed in dechlorinated water, blotted dry, then weighed and measured. Fork length in centimetres and weight to the nearest one-hundredth of a gram were recorded. Gill, liver and kidney were combined according to concentration and temperature. Composite samples of 7-10 organs were dried to constant weight for subsequent analysis of cadmium residues.

Mortality results were plotted on a probability scale against time on a logarithmic scale. ET50, slope of per cent mortality versus time and their 95% confidence limits were calculated according to Litchfield (1949).

The second experiment was carried out to determine sublethal effects of exposure to cadmium at the three temperatures of acclimation and exposure (6, 12 and 18°C). Changes in the cation constituents of the blood and effects on growth were investigated. Analysis of cadmium residues in gill, liver, kidney and whole fish were intended to give relative accumulation rates at the three temperatures.

Following the acclimation period, seventy fish were transferred randomly among the test tanks at each of the three test temperatures and starved for 24 hours before weighing. The trout in each test chamber were individually marked by clipping a part of the adipose, upper or lower caudal fins in various combinations of vertical and horizontal

clips. They were restrained for 30-45 seconds, then weighed and measured under red fluorescent lighting to minimize disturbance. No anaesthetic was used , since the restraint subdued the fish adequately for the measurement of length and weight.

Toxicant flow was begun 24 hours after the fish were returned to the test tanks. Exposure concentrations at 18°C ranged from 1-10 µg/l in a logarithmic series of five concentrations with one control. At 6 and 12°C the fish were exposed to five concentrations at logarithmic intervals from 1.8-18 µg Cd/l, with two controls at each temperature.

The fish were fed a mixture of no.3 and no.4 Ewos trout chow at a ration of five times the maintenance requirement for each temperature. Maintenance rations, determined by Kovacs (1979) for rainbow trout in the same apparatus, were 0.33% of body weight per day at 6°C, 0.53% per day at 12°C and 0.71% per day at 18°C. Tanks were siphoned daily before feeding and daily portions were divided into a morning and an afternoon feeding.

A second weighing was carried out at 10 days and rations were adjusted accordingly. After the full twenty-day exposure the fish were weighed and measured and blood samples were combined for all fish exposed to the same concentration and temperature. Five fish from each group were dissected to obtain gill, liver and kidney tissue, except the 18 µg/l group at 18°, in which only three fish survived. All fish were weighed before placing them in the drying oven to be dried to constant weight. Dry weights were taken to determine per cent dry weight and composite samples of five fish were blended to obtain homogeneous samples for analysis of cadmium residues in whole fish.

The third experiment was designed to follow mortality and changes in divalent ion concentrations of the plasma during a period of acute exposure, in an attempt to relate changes in ion concentrations with mortality. In addition, the response of fish acclimated to 12°C and exposed to cadmium at 6 and 18°C was compared to that of fish acclimated to the temperature of exposure in order to determine changes in response due to acclimation.

Three groups of ten fish were exposed to 0.3 mg Cd/l at each of the proposed temperature combinations of acclimation and exposure. One control group was also subjected to each temperature combination. All control groups were taken at the end of each period and regarded as 0 hours of exposure to cadmium. The schedule of exposure for the other three groups at the five temperature combinations is given in Table 2.

Table 2: Schedule of exposure time of rainbow trout exposed to 0.3 mg Cd/l at different acclimation and test temperatures

Test temperature (°C)	Acclimation temperature	Tank 1 (hours)	Tank 2 (hours)	Tank 3 (hours)
6	6	48	96	168
6	12	48	96	168
12	12	24	48	60
18	18	24	48	60
18	12	24	30	30

After the designated period of exposure at their respective acclimation and test temperatures, each group of trout was weighed, measured and sacrificed to obtain a composite blood sample. Magnesium and calcium concentrations were determined according to the method described previously. Mortality times (ET 50's) were taken from experiment one, when the fish were exposed at the temperature of acclimation. The two groups

that were acclimated at 12°C and exposed at 6 and 18°C were terminated when 50% mortality occurred so that enough remained to obtain a composite blood sample. Mortality times reported for these groups are the times at which fifty per cent mortality was observed.

Results and Analysis

Experiment 1-Acute response

Figure 2 shows the effect of acclimation and exposure temperature of 6, 12 and 18°C on the acute response of rainbow trout to a range of cadmium concentrations. The measured response was median effective time (ET50)-the time at which fifty per cent mortality was calculated at a given cadmium concentration.

It is apparent that fish acclimated to 6°C survived longer than fish acclimated and exposed at 12°C, which in turn survived longer than fish at 18°C. Median effective time, slope of per cent mortality versus time and their 95% confidence limits are presented in Table 3. Trout exposed at 6°C survived cadmium concentrations ranging from 0.1 to 3.2 mg/L for about seven days. ET50's in this concentration range were less than four days (80-90 hours) when the trout were acclimated and exposed at 12°C and at 18°C, ET50's ranged from 66-80 hours. Regression equations relating ET50's with cadmium concentration were calculated for assayed concentrations of 0.1 to 3.8 mg Cd/L at 6°C, 0.11 to 3.11 at 12°C and 0.03 to 3.29 at 18°C. ET50's calculated from these regressions are significantly greater in the range of 0.1 to 3.2 mg Cd/L at 6°C than at 12°C ($P < 0.01$) and 12°-acclimated and exposed fish survived the same concentration range for longer periods of time than at 18°C ($P < 0.10$).

Since median effective time is a measure of the rate at which cadmium affects the fish, a doubling or tripling of ET50 might be expected for every ten degree rise in temperature. Since the difference in ET50's between 12° and 18° is slight in comparison to the difference between 12° and 6°, the usual logarithmic temperature dependent response that is characteristic of poikilotherms subjected to rapid temperature changes is not apparent. It seems that

Table 3: Median effective time, slope of percent mortality plotted against time and their 95% confidence intervals of rainbow trout exposed to cadmium and acclimated at 6, 12 and 18°C

Temp. (°C)	Concentration (mg Cd/l)	No. of fish reacting	ET 50 (hours)	Slope
	3.29 (0.51)*	10	66 (57-77)**	1.27 (1.13-1.42)**
	0.89 (0.06)	10	72 (62-84)	1.27 (1.13-1.42)
18	0.33 (0.07)	10	82 (71-98)	1.22 (1.11-1.34)
	0.10 (0.02)	10	78 (67-91)	1.30 (1.16-1.46)
	0.03 (0.005)	10	76 (65-89)	1.29 (1.15-1.44)
	0.01 (0.005)	2		
	6.90 (0.31)	10	33 (27-40)	1.39 (1.20-1.61)
	3.11 (0.14)	10	80 (64-100)	1.44 (1.22-1.70)
12	1.04 (0.11)	9	80 (63-102)	1.44 (1.21-1.71)
	0.66 (0.07)	9	86 (71-105)	1.34 (1.17-1.54)
	0.11 (0.03)	9	90 (74-110)	1.35 (1.17-1.55)
	0.03 (0.003)	5		
	9.36 (0.46)	10	50 (43-58)	1.25 (1.14-1.38)
	3.80 (0.13)	10	140 (112-175)	1.45 (1.23-1.71)
6	1.02 (0.09)	7	150 (115-195)	1.50 (1.17-1.92)
	0.20 (0.04)	7	170 (136-213)	1.42 (1.16-1.73)
	0.10 (0.03)	9	170 (134-216)	1.45 (1.22-1.73)
	0.03 (0.002)	1		

* standard deviation ** 95% confidence intervals

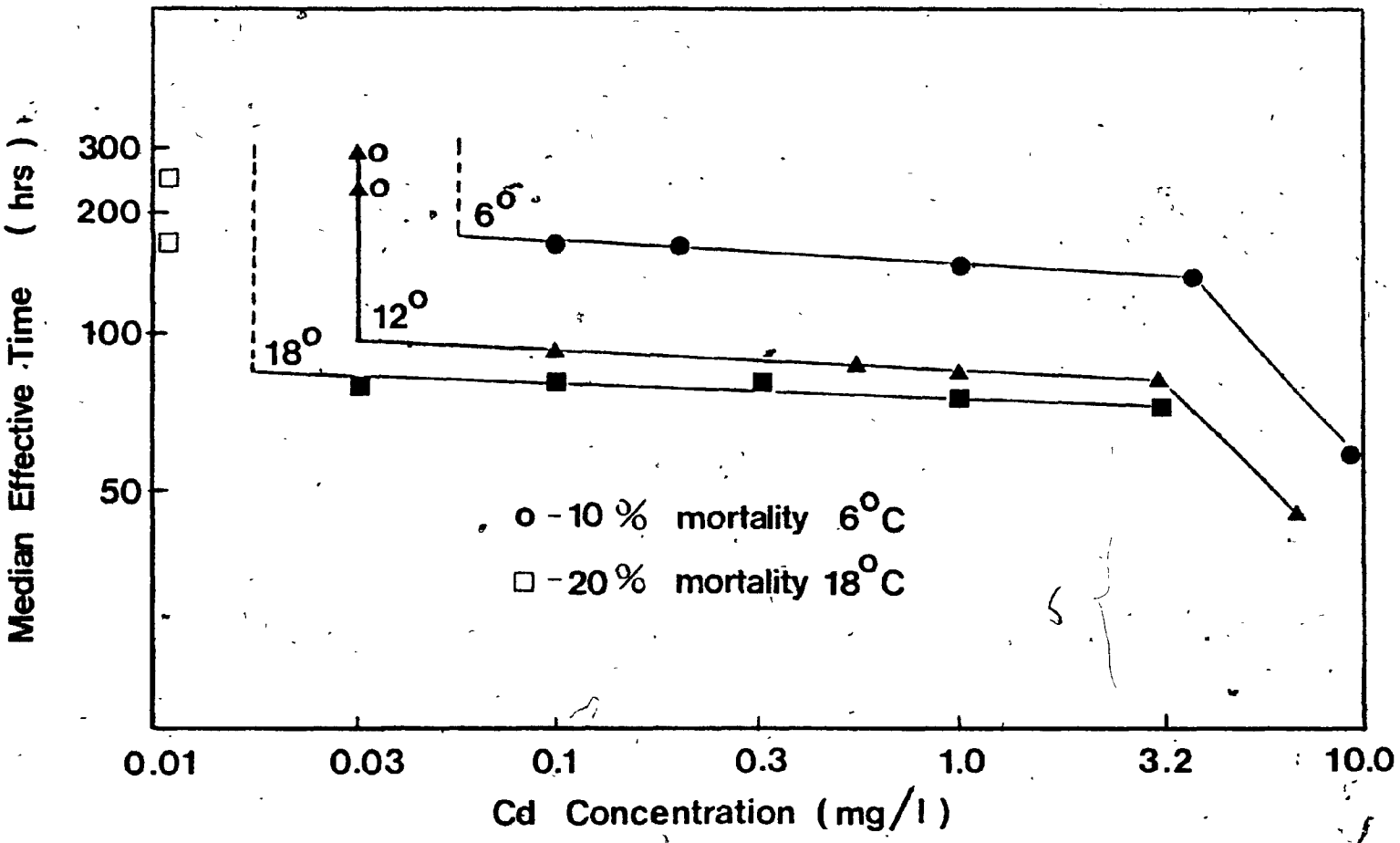


Figure 2: Median survival time of rainbow trout exposed to cadmium and acclimated to 6, 12 and 18°C.

acclimation has resulted in significant deviations in survival time from the response that could be predicted by temperature dependence.

The relationship between cadmium concentration and median effective time indicates very little change in ET50 over a thirty fold increase in concentration between 0.1 and 3.2 mg Cd/L at all three temperatures. Statistical analysis of the slope in this region (Figure 1) reveals no significant differences among the three temperatures ($P < 0.01$). Benoit et al. (1976) report a similar pattern with brook trout, Ball (1967) indicates a similar flat mortality curve with rainbow trout and Pascoe and Matthey (1977) indicate that the stickleback also shows this pattern in response to acute cadmium exposure.

The slope changes between 3.2 and 10.0 mg Cd/L indicating that a different mode of action may be operative at these high concentrations (see Sprague 1969). Ball (1967) demonstrated a similar change in the response of rainbow trout to high concentrations of cadmium above 1 mg/L. Pascoe and Matthey (1977) also reported a sharp increase in the rate of mortality of sticklebacks (Gasterosteus aculeatus) as the cadmium concentrations increased above 10 mg/L.

Ten day lethal threshold concentrations were between 10 and 30 ug Cd/L at 18°C, and between 30 and 100 ug Cd/L at 6°C. Ten day lethal threshold concentration at 12°C was 30 ug Cd/L. Ten day lethal thresholds therefore increased with decreasing temperature as did median survival time. The extent of mortality also varied with temperature. At 18°C, all fish died during the experimental period when exposed to concentrations ranging from 0.03 to 0.89 mg Cd/L and at 6°C, one to three fish survived at concentrations ranging from 0.1 to 1.0 mg Cd/L for fourteen days.

Symptoms of poisoning began with erratic and violent swimming followed by lethargy and loss of equilibrium. The fish became dark in appearance and finally sank to the bottom. The fish seemed to be hypersensitive to disturbance. On several occasions, fish that were taken out of the tanks showed no response to handling and were no longer breathing but exhibited rhythmic contractions of the body musculature and tetanic extensions of the jaw.

Cadmium residues in fish tissues at mortality (Figure 3) demonstrate that accumulation of cadmium is a function of the concentration of exposure, although the trend is less pronounced in whole fish. At the two extreme temperatures, cadmium levels at mortality were similar, however, since mortality occurred much later in fish exposed at 6°C, the rate of accumulation is much slower at 6° than at 18°C. The comparable residues at mortality for the two extreme temperatures indicate that a certain level of cadmium in tissues is associated with a particular concentration of exposure. The relationship is most evident in gill tissue residues. The amount of cadmium in all tissues increases with the concentration of cadmium to which the fish are exposed.

Since the change in survival time is small in the range of 0.1–3.2 mg Cd/l, it appears that rainbow trout can accumulate greater levels of cadmium with correspondingly little change in susceptibility. If cadmium does cause damage to one of these tissues resulting in mortality, greater quantities of cadmium seem to be rendered inactive with increasing cadmium concentration either by a physical or a physiological process within the fish tissues. It is evident that no single critical level of cadmium residues in the tissues examined can be correlated with mortality for all the concentrations of exposure.

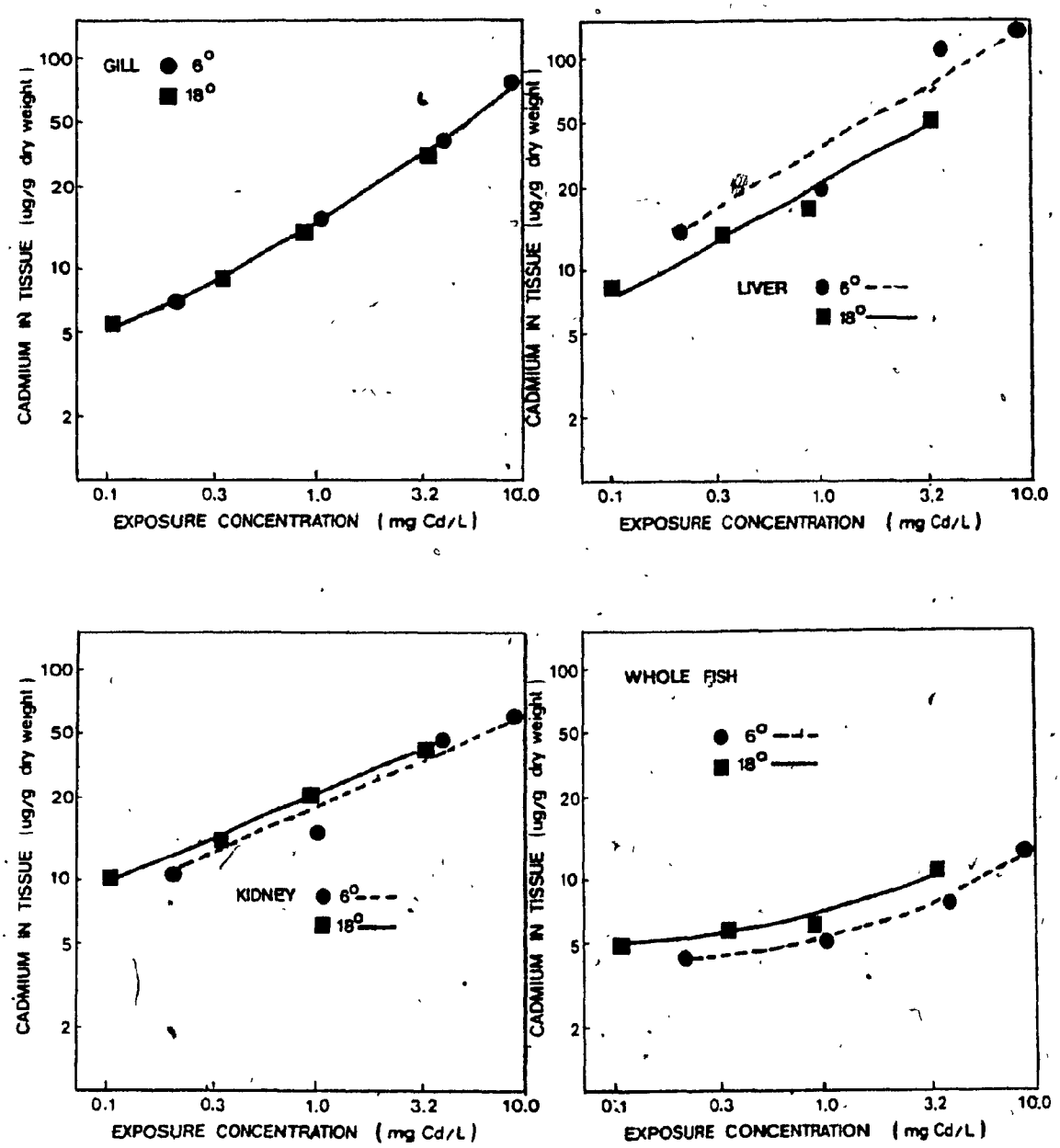


Figure 3: Cadmium residues in gill, liver, kidney and whole rainbow trout at mortality when exposed and acclimated at 6 and 18°C. Lines drawn were fitted by eye.

Experiment 2-Chronic effects

During the twenty-day sublethal exposure the rainbow trout consumed their food rations readily except at some of the higher concentrations when certain fish became lethargic and did not respond to feeding. One death occurred at 18° and 10 µg Cd/L, seven at 12° and 18 µg Cd/L and one at 6° and 18 µg Cd/L.

Growth results in terms of per cent wet weight gain are presented in Table 4. Analysis of variance showed no significant effect due to cadmium exposure at any of the three temperatures. However, comparison of mean per cent wet weight gains at some of the higher cadmium concentrations with the controls shows a marked reduction in growth and a great deal of variance in individual per cent wet weight gains at the higher cadmium concentrations is evident. The growth rate increased with temperature and this increase is primarily due to the size of the food ration, which was proportionate to temperature. The fish were fed approximately 1%, 2% and 3% per day above their maintenance requirements at 6, 12 and 18°C respectively and the rations are reflected in the growth rates.

Dry weight proportions are given in Table 5 and analysis of variance indicates no significant difference among treatments ($P < 0.05$). Conversion of growth results from wet weight gains to dry weight gains would not have changed the growth results. Any damage to gills or kidneys was not severe enough to cause an appreciable change in the water balance within the fish as a result of exposure to cadmium.

There does appear to be a slight increase in per cent dry weight with temperature and this increase may be due to the proportionately greater weights attained by the fish at high temperatures. Love (1970 p.89) points out that water content decreases with age and size of fish.

Table 4: Per cent wet weight gain of rainbow trout exposed to sublethal concentrations of cadmium for twenty days.

Temp. (°C)	Conc. (ug Cd/l)	Mean % wet weight gain-10 days	Mean % wet weight gain-20 days	n	Analysis of variance
	9.2 (1.4)*	13.81 (4.92)**	44.08 (8.27)**	9	
	5.5 (0.4)	24.79 (1.41)	62.49 (2.38)	10	F = 1.50
18	4.0 (0.7)	21.84 (3.67)	56.70 (7.81)	10	F (0.05);5,53 =2.4
	2.0 (0.5)	21.36 (4.51)	57.12 (4.66)	10	
	1.5 (0.5)	27.12 (1.89)	63.64 (2.34)	10	
	Control	23.54 (2.21)	60.76 (5.87)	10	
	17.5 (1.2)	14.21 (4.37)	30.50 (8.30)	3	
	10.6 (0.8)	16.70 (3.03)	33.51 (5.45)	10	
12	5.5 (0.8)	14.56 (2.11)	36.56 (3.85)	10	F = 1.34
	4.2 (0.6)	18.98 (1.27)	42.29 (3.29)	10	F (0.05);6,56 = 2.28
	2.0 (0.6)	19.86 (1.41)	44.65 (3.49)	10	
	Control 1	17.20 (2.02)	40.30 (3.62)	10	
	Control 2	20.68 (3.02)	42.69 (3.11)	10	
	18.8 (0.4)	8.11 (1.49)	20.80 (1.91)	9	
	10.0 (0.7)	10.65 (1.56)	21.44 (2.77)	10	
6	6.1 (0.2)	8.52 (1.37)	23.69 (1.60)	10	F = 0.64
	4.2 (0.4)	11.91 (1.39)	24.14 (2.42)	10	F (0.05);6,62 = 2.25
	1.8 (0.1)	15.47 (0.81)	24.76 (1.15)	10	
	Control 1	11.98 (0.68)	23.72 (1.55)	10	
	Control 2	11.70 (1.29)	24.81 (1.31)	10	

* Standard deviation

** Standard error

Table 5: Per cent dry weight of rainbow trout after twenty days exposure to sublethal concentrations of cadmium

Temp. (°C)	Mean cadmium concentration (µg/l)	Mean % dry weight	Standard deviation	n	Analysis of variance
18	9.2	24.48	2.69	9	F = 0.85 F(0.05); 5,53 = 2.40
	5.5	25.14	1.39	10	
	4.0	24.02	1.72	10	
	2.0	25.18	0.90	10	
	1.5	24.74	0.90	10	
	Control	24.44	0.91	10	
12	17.5	23.93	0.44	3	F = 1.08 F(0.05); 6,56 = 2.28
	10.6	23.32	1.23	10	
	5.5	23.75	1.08	10	
	4.2	23.70	0.72	10	
	2.0	23.29	1.06	10	
	Control 1	23.34	0.59	10	
	Control 2	22.78	1.42	10	
6	18.8	23.70	1.14	9	F = 1.07 F(0.05); 6,62 = 2.25
	10.0	23.72	1.11	10	
	6.1	24.04	1.30	10	
	4.2	23.18	0.50	10	
	1.8	23.14	0.49	10	
	Control 1	22.98	1.08	10	
	Control 2	22.83	0.98	10	

Effects on blood ion concentrations

Calcium concentrations in the blood of rainbow trout increased sharply as a function of cadmium concentration at both 12 and 18°C (Fig. 4). Ion concentrations were first subjected to analysis of variance and a significant difference among groups ($P < 0.05$) was used as a criterion for further analysis using a 't' test in comparison to controls. Results of calcium ion analysis are presented in Table 6. At 18° a significant decrease occurred at 4.0 µg Cd/l ($P < 0.05$) and highly significant increases at both 5.5 and 9.2 µg Cd/l ($P < 0.01$). At 12° the effect was less pronounced but significant differences from the two controls occur at 5.5, 10.6 and 17.5 µg Cd/l ($P < 0.01$). In both instances the increase in calcium concentrations occurs at very low cadmium concentrations. Exposure to cadmium at 6°C resulted in elevations of the blood calcium concentration that were not strictly related to cadmium concentration, with a maximum effect at the intermediate concentration of 6.1 µg Cd/l. This unusual relationship may be the result of a short term mechanical failure of the 6° water supply during which the toxicant reached the tanks undiluted, resulting in elevated concentrations of cadmium over a period of several hours. Statistical comparison of exposed groups with controls reveals that significant increases ($P < 0.01$) in calcium concentrations of the blood occurred at all exposure concentrations ranging from 4.2 to 18.8 µg Cd/l.

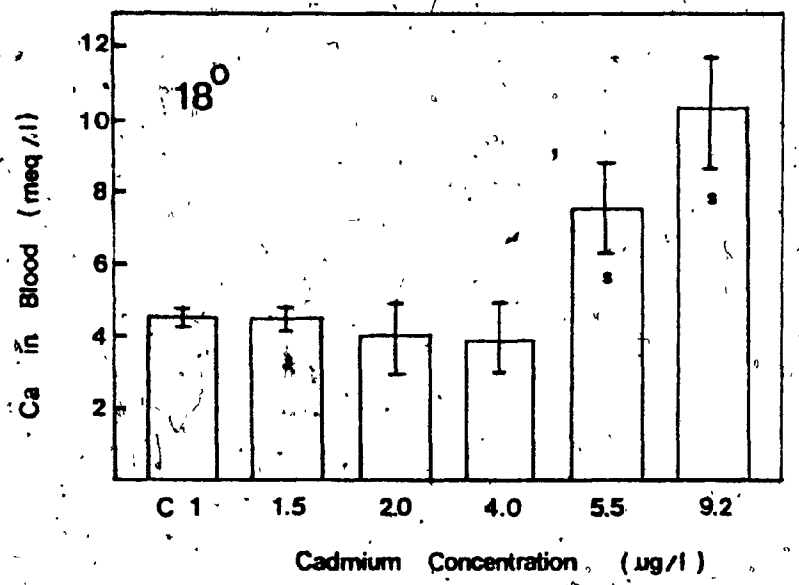
Magnesium concentrations in the blood are more constant than calcium concentrations with respect to exposure concentration (Figure 5). Table 7 indicates that a significant increase occurred at 9.2 µg Cd/l and 18° ($P < 0.05$), 10.6 and 17.5 ug Cd/l at 12° ($P < 0.01$). At 6° significant increases occurred at 6.1 µg Cd/l ($P<0.05$) and 18.8 µg Cd/l.

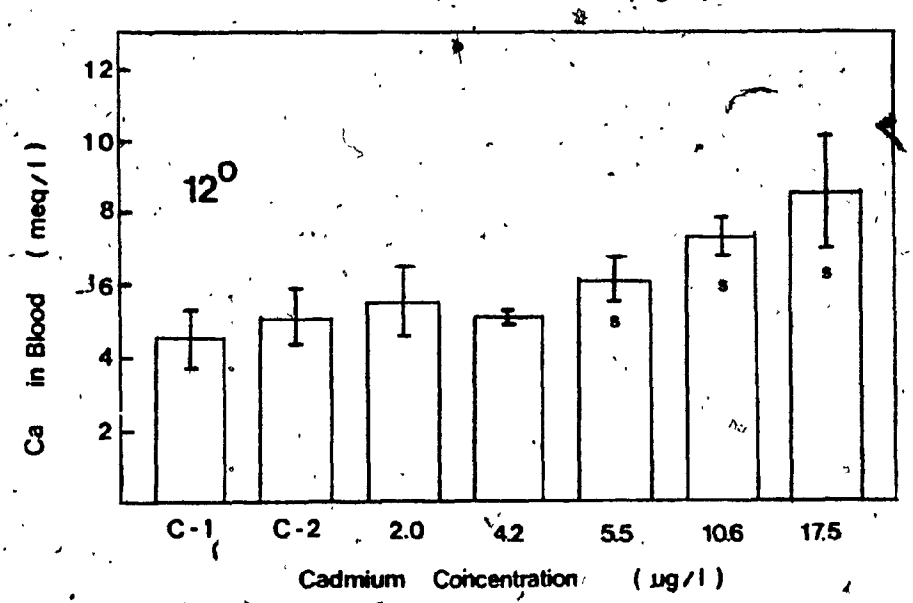
Table 6 : Calcium concentrations in the blood of rainbow trout after twenty days sublethal exposure to cadmium.

([a] 95% confidence limits ; * P < 0.05 ; ** P < 0.01)

	Mean cadmium concentration (µg/l)	Number of fish in sample	Mean Ca in blood (meq/l)	t value in comparison to Control 1	t value in comparison to Control 2
	C-1	10	4.7 ± 0.25[a]		
	1.5	10	4.6 ± 0.43	0.88	
18°	2.0	10	4.1 ± 1.03	2.41	
	4.0	10	3.9 ± 1.14	2.89*	
	5.5	10	7.6 ± 1.18	10.47**	
	9.2	9	10.4 ± 1.49	16.52**	
	C-1	10	4.5 ± 0.76		
	C-2	10	5.1 ± 0.75		
	2.0	10	5.6 ± 0.94	3.89*	1.79
12°	4.2	10	5.2 ± 0.14	3.85*	0.57
	5.5	10	6.2 ± 0.57	7.59**	5.02**
	10.6	10	7.4 ± 0.52	13.36**	10.85**
	17.5	3	8.6 ± 1.72	9.38**	8.05**
	C-1	10	3.2 ± 0.14		
	C-2	10	3.1 ± 0.25		
	1.8	10	3.3 ± 0.25	1.49	2.19
6°	4.2	10	4.6 ± 0.50	11.63**	11.65**
	6.1	10	5.2 ± 0.29	25.81**	23.33**
	10.0	10	3.8 ± 0.50	4.98**	5.43**
	18.8	9	3.9 ± 0.43	6.74**	7.04**

Figure 4: Mean calcium concentrations in the blood of rainbow trout exposed to sublethal concentrations of cadmium for twenty days and acclimated to 6, 12 and 18°C. Bars represent 95% confidence limits and 's' represents a significant difference compared to both controls ($P < 0.01$).


18°
Ca in Blood (meq/l)
12
10
8
6
4
2
C 1
1.5
2.0
4.0
5.5
9.2
Cadmium Concentration (µg/l)


12°
Ca in Blood (meq/l)
12
10
8
6
4
2
C-1
C-2
2.0
4.2
5.5
10.6
17.5
Cadmium Concentration (µg/l)

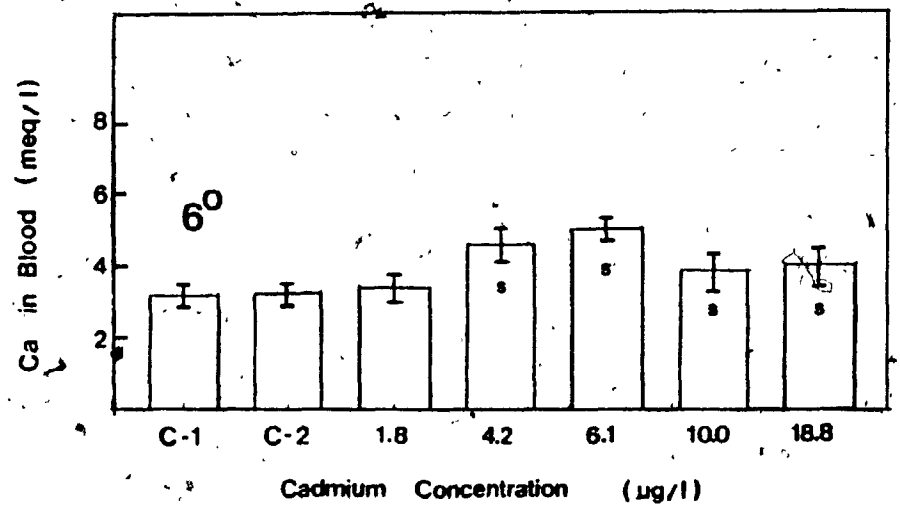
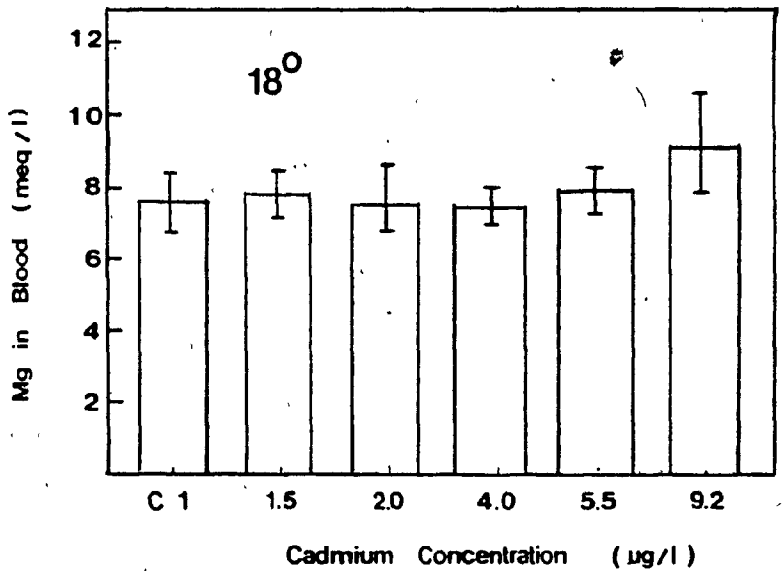

6°
Ca in Blood (meq/l)
8
6
4
2
C-1
C-2
1.8
4.2
6.1
10.0
18.8
Cadmium Concentration (µg/l)

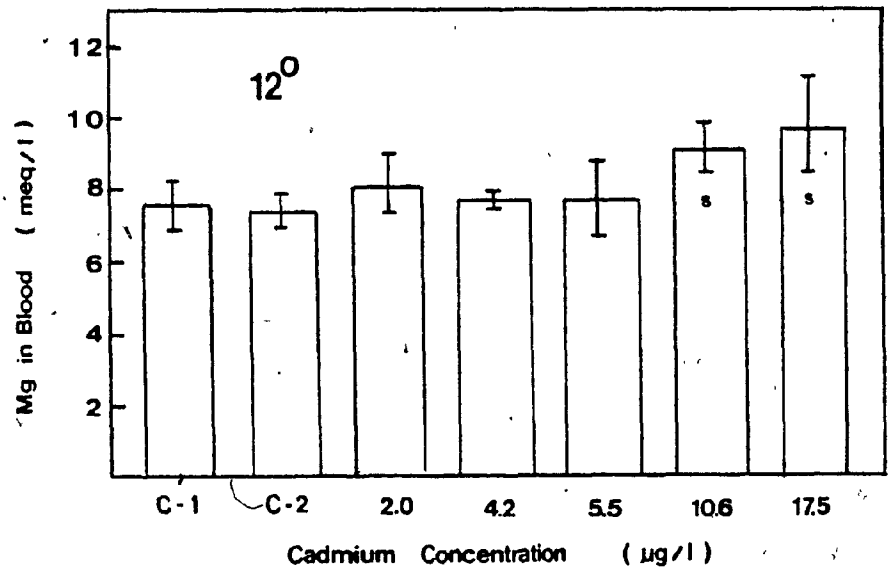
Table 7: Magnesium concentrations in the blood of rainbow trout after twenty days exposure to cadmium. (*P<0.05; **P<0.01)

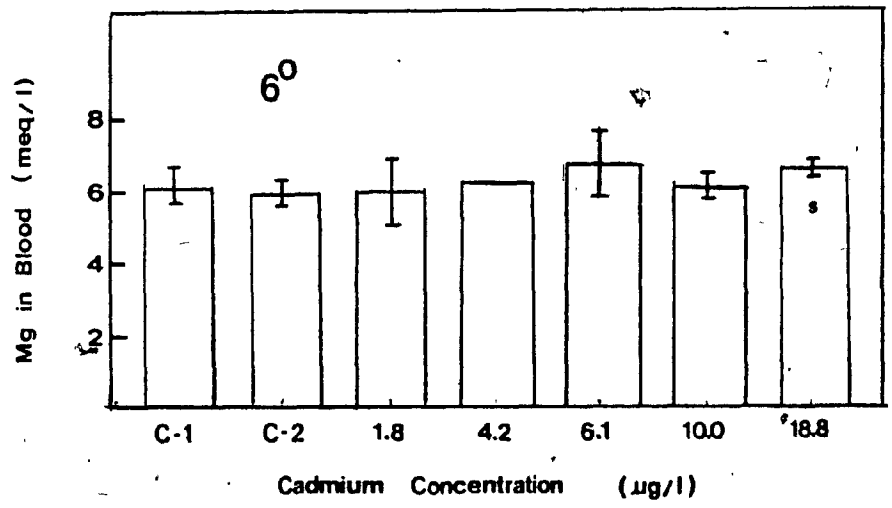
	Mean Cd concentration (ug/l)	Mean Mg in blood (meq/l)	t value in comparison to control 1	t value in comparison to control 2
	Control 1	7.6 ± 0.90[a]		
	1.5	7.8 ± 0.66	0.78	
18°	2.0	7.6 ± 1.12	-	
	4.0	7.5 ± 0.50	0.42	
	5.5	7.9 ± 0.62	1.18	
	9.2	9.2 ± 1.41	4.11*	
	Control 1	7.3 ± 0.38		
	Control 2	7.5 ± 0.66		
	2.0	7.9 ± 0.86	2.73	1.59
12°	4.2	7.6 ± 0.15	3.22*	0.65
	5.5	7.6 ± 0.97	1.24	0.37
	10.6	9.0 ± 0.72	9.02**	6.68**
	17.5	9.6 ± 1.39	6.87**	5.89**
	Control 1	5.9 ± 0.30		
	Control 2	6.1 ± 0.43		
	1.8	6.0 ± 1.00	0.41	0.40
6°	4.2	6.3 ± -	5.77**	2.04
	6.1	6.8 ± 1.00	3.73*	2.80*
	10.0	6.1 ± 0.43	1.66	0.00
	18.8	6.7 ± 0.14	10.32**	5.77**

[a] 95 % confidence limits

Figure 5: Mean magnesium concentrations in the blood of rainbow trout exposed to sublethal concentrations of cadmium for twenty days and acclimated to 6, 12 and 18°C. Bars represent 95% confidence intervals and 's' represents a significant difference compared to both controls ($P < 0.01$).


18°
Mg in Blood (meq/l)
12
10
8
6
4
2
C 1
1.5
2.0
4.0
5.5
9.2
Cadmium Concentration (µg/l)


12°
Mg in Blood (meq/l)
12
10
8
6
4
2
C-1
C-2
2.0
4.2
5.5
10.6
17.5
s
s
Cadmium Concentration (µg/l)


6°
Mg in Blood (meq/l)
8
6
4
2
C-1
C-2
1.8
4.2
6.1
10.0
18.8
s
Cadmium Concentration (µg/l)

Sodium and potassium concentration in the blood were determined for trout exposed to cadmium at 12°C (Figure 6,Table 8). Although potassium concentration did not show a significant difference by analysis of variance ($P < 0.05$), sodium concentrations were significantly higher than controls at the highest cadmium concentration of 17.5 µg/l ($P < 0.05$).

The pronounced increase in calcium concentrations of the blood appears to be a very specific effect of cadmium exposure since the other ion concentrations remained relatively stable. The response is apparent at all three temperatures and calcium elevations were most pronounced at 18°C. The toxic effects of cadmium seem to be influenced markedly by temperature during chronic exposure as they are during acute exposure.

Cadmium residues in tissues

Analysis of cadmium residues in tissues following the twenty day sublethal exposure revealed a temperature dependent rate of accumulation as was evident in the first experiment (Figures 3 and 7). Cadmium had accumulated to greater levels in all tissues of fish exposed at 18° as compared to 12° and 6°C. There was some accumulation in tissues of fish exposed at 12° and slight or negligible accumulation of cadmium at 6°C.

Gill and kidney tissue accumulated the most cadmium. The absolute quantities of cadmium, on a dry weight basis, in these tissues were as much as one thousand times as great as the concentrations of cadmium in the water to which the fish had been exposed. These organs appear to be the primary sites at which cadmium accumulates.

Liver and whole fish accumulated lesser quantities of cadmium and demonstrated the same concentration and temperature related pattern as gill and kidney tissue. Residues in both tissues at 6° were only slightly greater than controls and at 12 and 18° significant elevations occur.

Table 8: Sodium and potassium concentrations in the blood of rainbow trout exposed to cadmium at 12°C for twenty days

* $P < 0.05$; ** $P < 0.01$

Mean cadmium concentration (µg/l)	Mean sodium in blood (meq/l)	95% confidence limits	t value compared to Control 1	t value compared to Control 2
Control 1	74.6	± 6.93		
Control 2	69.0	± 2.98		
2.0	71.2	± 9.32	1.26	0.97
4.2	71.1	± 5.97	1.65	1.42
5.5	73.1	± 5.92	0.71	2.66
10.6	68.3	± 6.36	2.89	0.43
17.5	84.9	± 7.85	4.24*	8.15**

Mean cadmium concentration (µg/l)	Mean potassium in blood (meq/l)	95% confidence limits	Analysis of variance
Control 1	26.5	± 0.99	
Control 2	29.1	± 1.44	
2.0	27.2	± 1.79	$F = 1.99$
4.2	28.3	± 3.01	$F_{.05;\ 6,14} = 2.85$
5.5	25.5	± 2.26	
10.6	31.4	± 3.50	
17.5	26.3	± 2.61	

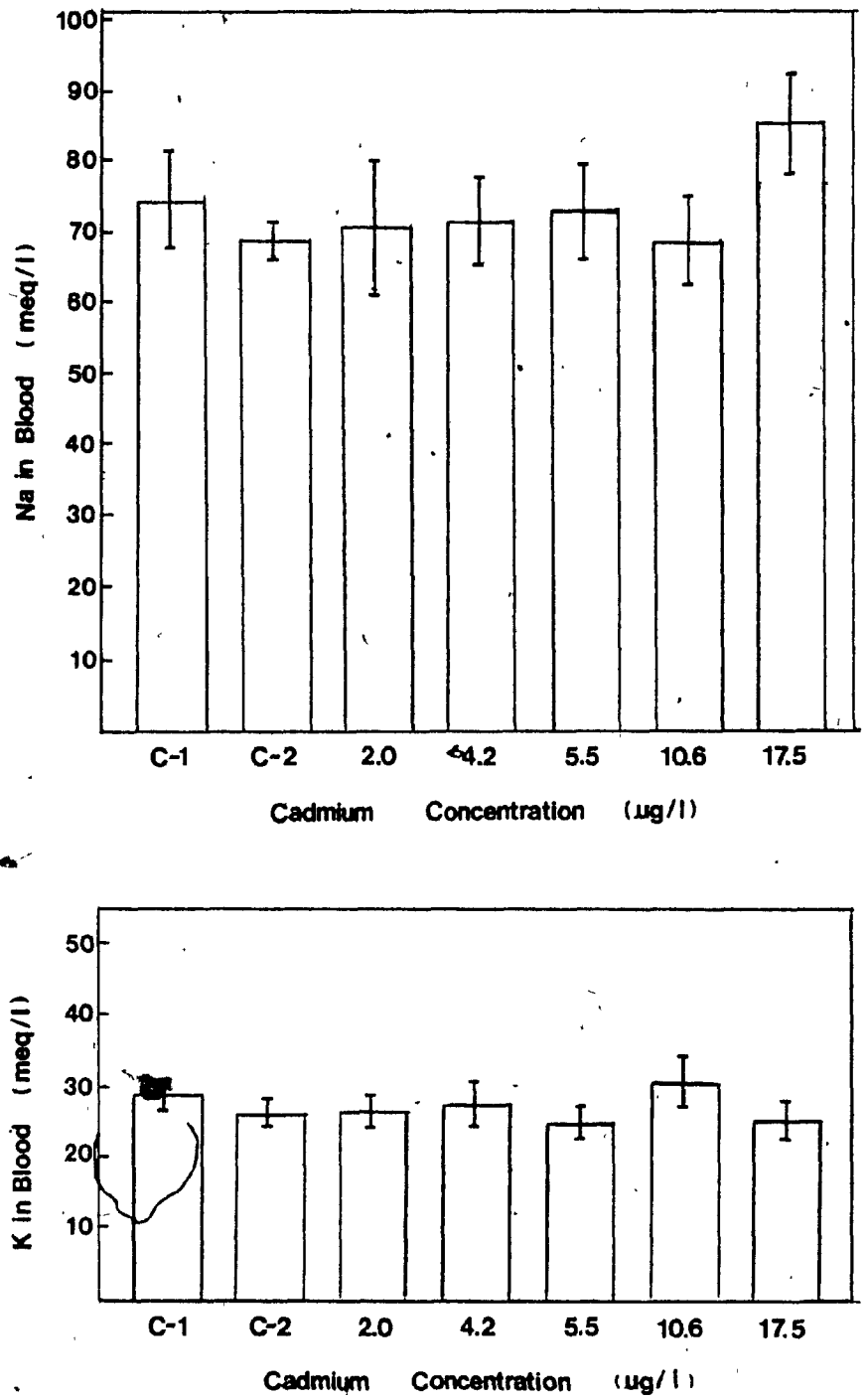


Figure 6: Mean sodium and potassium concentrations in the blood of rainbow trout exposed to sublethal concentrations of cadmium for twenty days and acclimated to 12°C. Bars represent 95% confidence intervals.

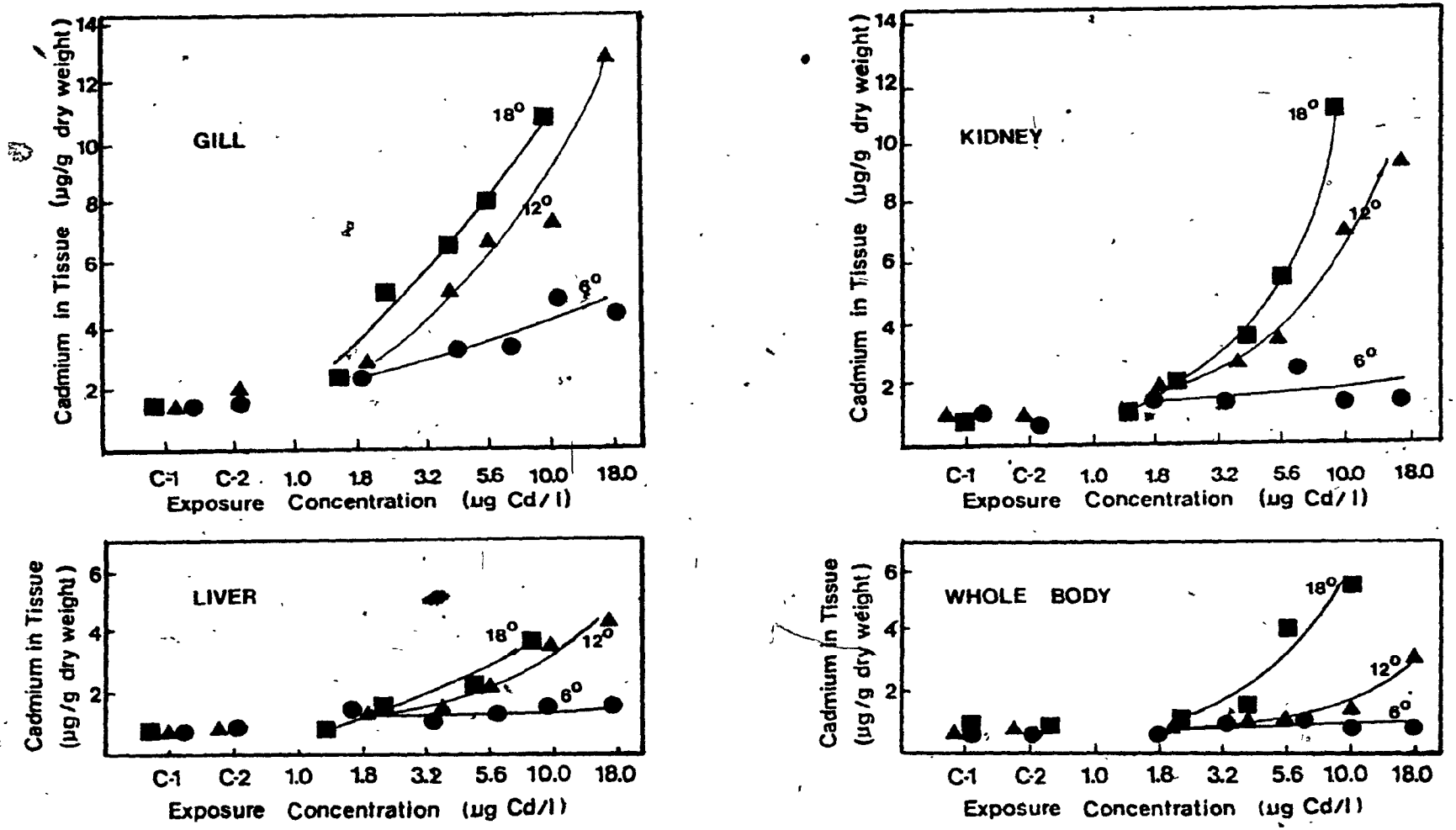


Figure 7: Cadmium residues in gill, kidney, liver and whole rainbow trout exposed to sublethal concentrations of cadmium for twenty days and acclimated to 6, 12 and 18°C. Squares represent 18°, triangles-12° and circles-6°C.

Experiment 3

Figure 8 shows the variation in plasma calcium concentrations with time of exposure to 0.3 mg Cd/l. The response of fish acclimated to 12° and exposed at 6 and 18° is presented along with those of trout acclimated and exposed at 6, 12 and 18°C. Calcium concentrations at various intervals of exposure are given for each temperature regime in Table 9. Individual results of calcium concentration in plasma and the time at which they occurred were related by a regression equation to determine the time at which the calcium level in the plasma was reduced to 3.0 meq/l. Ninety-five per cent confidence limits for these times were calculated according to Sokal and Rohlf (1969 p.446). The arbitrary concentration of 3.0 meq/l was selected as a basis for comparison with time to fifty per cent mortality because in all cases calcium concentrations were below this level when mortality had occurred.

It is apparent in all cases that calcium concentrations in the plasma declined steadily with time of exposure to 0.3 mg Cd/l. The rate of decrease was greatest when the trout were acclimated to 12° and exposed to cadmium at 18°. The rates of decrease are similar when fish were acclimated and exposed at 12 or 18° and were least when the fish were exposed at 6°C.

It is apparent from Figure 9 that median effective time and the time at which the calcium concentrations were reduced to 3.0 meq/l are closely related. Fish acclimated to 12° and exposed to cadmium at 6, 12 and 18° show an inverse temperature dependence of survival time with a Q_{10} of 3.98. Calcium reduction times show the same inverse temperature dependence with a similar Q_{10} of 3.88. When fish were acclimated to test temperatures a significant deviation from strict temperature dependence

Table 9: Change of calcium concentration in the plasma of rainbow trout with time of exposure to 0.3 mg Cd/l and the variation with exposure (E) and acclimation (A) temperature. * 95% confidence limits. ** time of calcium reduction to 3.0 meq/l and its 95% confidence limits.

Temp.	Mean Cd conc. mg/l S.D.	Exposure time (hours)	Ca Conc. in plasma (meq/l)	Regression of Ca in plasma with time of exposure	Ca reduction time (hours)
E-18°C A-12°C	0.28 ± .04	0 24 30	5.26 ± 0.59 3.58 ± 0.26 2.69 ± 0.13	Y =5.305-0.081X	28 ± 9**
E-18°C A-18°C	0.33 ± .02	0 24 48 60	5.12 ± 0.79 3.87 ± 0.15 3.42 ± 0.26 2.76 ± 0.28	Y =5.007- 0.036X	56 ± 14
E-12°C A-12°C	0.30 ± .04	0 24 48 60	5.26 ± 0.53 3.84 ± 0.48 3.33 ± 0.20 2.85 ± 0.26	Y =5.083- 0.038X	55 ± 13
E- 6°C A-12°C	0.31 ± .02	0 48 96 168	5.22 ± 0.70 3.83 ± 0.31 3.46 ± 0.24 2.95 ± 0.15	Y =4.849-0.013X	142 ± 57
E- 6°C A- 6°C	0.34 ± .02	0 48 96 168	4.28 ± 0.52 3.49 ± 0.39 3.30 ± 0.56 2.88 ± 0.22	Y =4.095- 0.0078X	140 ± 57

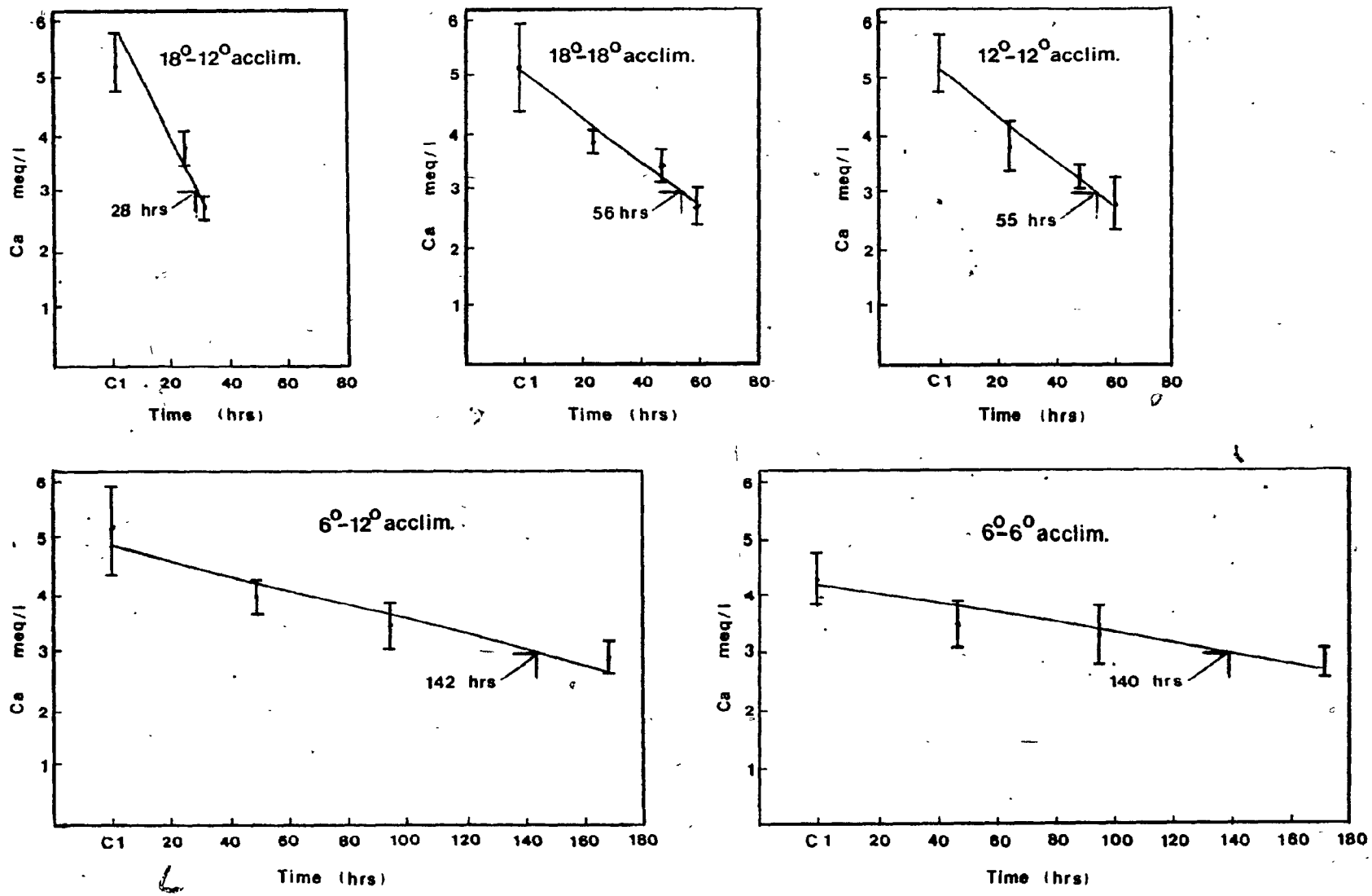


Figure 8: Change in the calcium concentrations in the plasma of rainbow trout with time of exposure to 0.3 mg Cd/l; variation with temperature of exposure and acclimation.

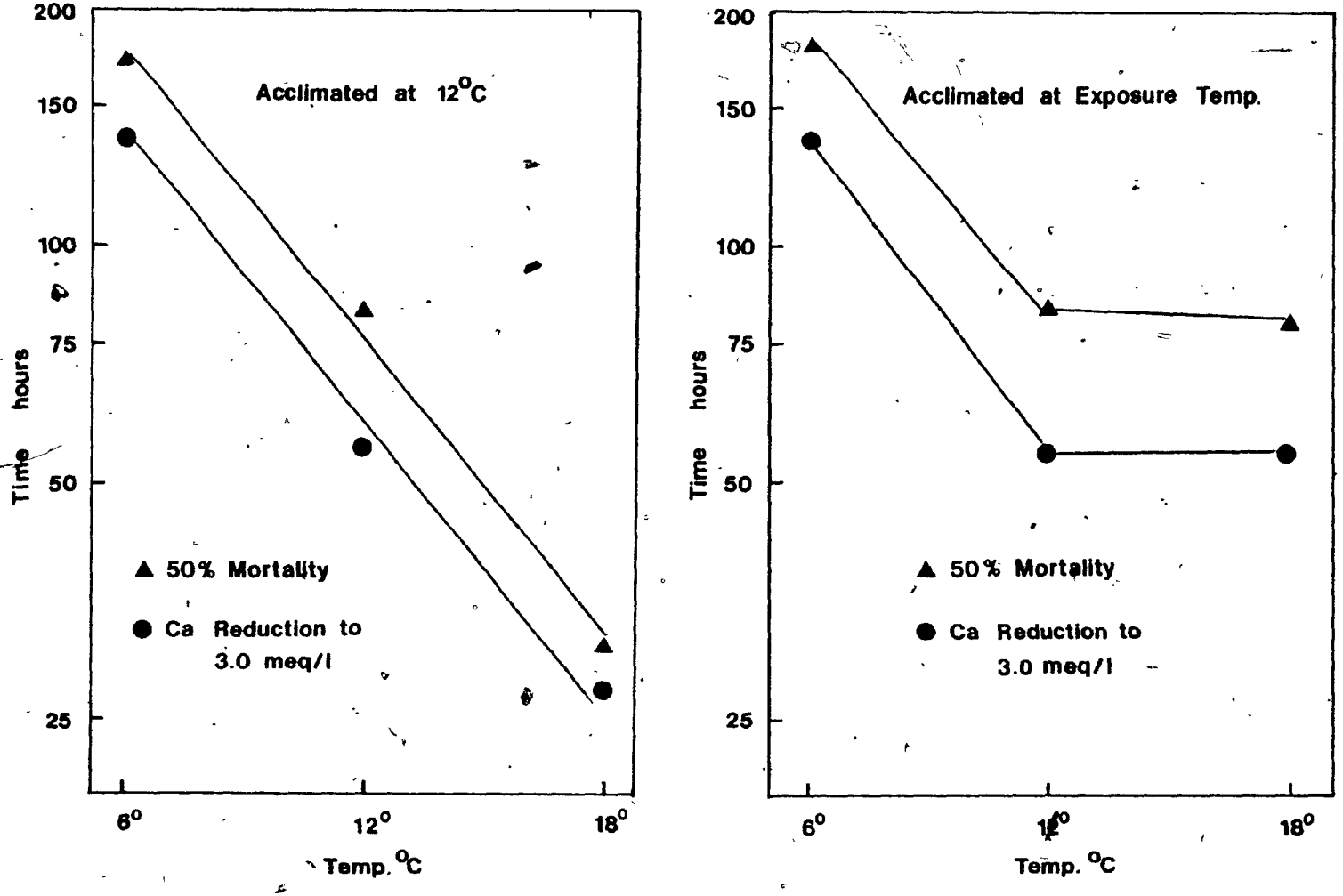


Figure 9: Time to 50% mortality and calcium reduction time at three temperatures of rainbow trout exposed to 0.3 mg Cd/l. A. Acclimated to 12°C. B. Acclimated to exposure temperature.

is evident in the case of fish acclimated to 18°C. Both calcium reduction and survival time are considerably greater than when 12° acclimated trout are exposed at 18°. Warm acclimation results in very little difference in resistance or calcium reduction time between 12 and 18°C.

Magnesium concentrations in plasma (Table 10) increased with time of exposure to cadmium but do not show as consistent a pattern as calcium concentrations. While calcium concentrations declined steadily, magnesium levels generally reached a maximum before the final sampling time and in some cases declined again. Little change in magnesium concentration occurred at 6°C.

Plasma osmolality was determined in all blood samples and results are presented in Table 11. Despite the disturbance in the concentrations of magnesium and calcium , no effect on osmolality was evident. Analysis of variance indicates that the differences in osmolality are not significant ($P < 0.05$). The opposing shifts in magnesium and calcium concentrations result in fairly constant total divalent ion concentrations which is not manifested as a change in osmolality.

Table 10: Change of magnesium concentrations in the plasma of rainbow trout with time of exposure to 0.3 mg Cd/l and the variation with temperature of acclimation and exposure.

Temperature E- exposure A- acclimation	Cd concentration (mg/l) Mean ± S.D.	Time of exposure (hours)	Mean and 95% confidence limits of Mg in plasma (meq/l)
E-18°C A-12°C	0.28 ± 0.04	0	2.14 ± 0.27
		24	3.81 ± 0.28
		30	3.39 ± 0.09
E-18°C A-18°C	0.33 ± 0.02	0	2.29 ± 0.14
		24	2.93 ± 0.21
		48	3.72 ± 0.17
		60	3.95 ± 0.12
E-12°C A-12°C	0.30 ± 0.04	0	2.13 ± 0.05
		24	2.86 ± 0.08
		48	2.61 ± 0.13
		60	2.78 ± 0.10
E- 6°C A-12°C	0.31 ± 0.02	0	2.23 ± 0.16
		48	1.92 ± 0.10
		96	2.34 ± 0.06
		168	2.31 ± 0.07
E- 6°C A- 6°C	0.34 ± 0.02	0	1.92 ± 0.19
		48	1.84 ± 0.17
		96	2.34 ± 0.06
		168	2.46 ± 0.24

Table 11: Plasma osmotic concentrations of rainbow trout exposed to 0.9 mg Cd/l for the given lengths of time under the temperature regimes specified.

Temperature E- exposure A- acclimation	Time of exposure (hours)	Plasma osmotic concentrations (mosm/l) Mean ± S.D.	Analysis of variance
	0	301 ± 9.6	
E-18°C	24	291 ± 5.6	F = 0.63
A-12°C	30-1	299 ± 15.0	F (.05); 3,8 = 4.07
	30-2	294 ± 8.0	
	0	295 ± 4.4	
E-18°C	24	303 ± 2.9	F = 1.67
A-18°C	48	303 ± 4.7	F (.05); 3,8 = 4.07
	60	294 ± 4.0	
	0	289 ± 5.5	
E-12°C	24	300 ± 10.8	F = 1.32
A-12°C	48	287 ± 8.7	F (.05); 3,8 = 4.07
	60	294 ± 8.0	
	0	297 ± 4.7	
E- 6°C	48	288 ± 6.4	F = 3.65
A-12°C	96	294 ± 4.0	F (.05); 3,8 = 4.07
	168	301 ± 4.0	
	0	291 ± 5.6	
E- 6°C	48	301 ± 4.0	F = 3.53
A- 6°C	96	302 ± 4.5	F (.05); 3,8 = 4.07
	168	296 ± 3.6	

Discussion

Changes in acclimation temperature influenced both survival time and incipient lethal concentration when the rainbow trout were exposed to acute concentrations of cadmium. The inverse relationship between survival time and temperature is a common observation when fish are exposed to toxic substances (Lloyd 1960, Brown et al. 1967, Hodson and Sprague 1975, MacLeod and Pessah 1973). Higher temperatures result in greater ventilatory and cardiac rate (Roberts 1973) and pollutants may enter the gill tissue and the blood more rapidly at higher temperatures.

The high levels of cadmium in the gill tissue at mortality would indicate that this is the primary site of entry. The rainbow trout accumulated cadmium more quickly in all tissues at the higher temperatures and it appears that the rate of mortality increases with temperature because of increased rate of entry and transport of cadmium to vital organs.

Since the fish were relatively inactive during the acute exposure, resting metabolic rate as a function of acclimation temperature might provide a good indication of the rate at which cadmium causes mortality. Evans et al. (1962) report an approximate doubling of the resting metabolic rate of rainbow trout acclimated to 16°C as compared to that of trout acclimated to 8°C. A similar doubling of the standard metabolic rate of rainbow trout is reported between 5 and 15° by Dickson and Kramer (1971).

Rate of mortality (inverse of survival time) of rainbow trout exposed to cadmium in this study approximately doubles in a similar

range of 6-18°C. The rate of mortality appears to be related to the changes in metabolic activity that occur with changes in temperature.

Incipient lethal concentration was inversely related to temperature. Not only did the fish survive longer at 6° than at high temperatures, but they also resisted higher concentrations of cadmium. Lethal thresholds may depend on rate of entry, sensitivity and the rate of elimination and detoxification of cadmium and they may be effected to varying degrees by metabolic changes that occur with change in acclimation temperature.

Several investigators have demonstrated induction of metal binding proteins as a result of exposure to cadmium and they believe the synthesis of this protein to be a detoxification mechanism (Colucci et al. 1975, Kimura et al. 1974). Olafson and Thompson (1974) showed an increase of the cadmium-binding protein in the liver of the copper rock fish (Sebastodes caurinus) with increasing dose of cadmium and Marafante (1975) has demonstrated the existence of the protein in the liver and kidney of goldfish (Carassius auratus L.) .

Evans et al. (1962) report that liver metabolic rates of 8°-acclimated rainbow trout are higher than those of 16°-acclimated trout. If rainbow trout also produce cadmium-binding protein in liver tissue, the higher metabolic rates of cold-acclimated liver tissue may allow a more active detoxification mechanism at low temperature. The higher levels of cadmium in liver tissue of cold-acclimated rainbow trout in this experiment may be the result of more efficient detoxification at lower temperatures. This hypothesis would explain the higher lethal thresholds that were tolerated by cold-acclimated rainbow trout.

The negligible concentration dependent response that is observed between 0.1 and 3.2 mg Cd/l indicates that the trout are somehow capable

of dealing with increasing concentrations with relatively little change in response time. Cadmium residues in gill, liver and kidney indicate that the fish accumulated the metal in proportion to the concentration of cadmium to which they were exposed. It seems that a process occurs which detoxifies greater amounts of cadmium as the exposure concentration increases.

Bryan and Hidalgo (1976) report a correlation between the synthesis of cadmium binding protein and the content of cadmium in rat liver nuclei and have reported increased synthesis of this protein with greater doses of cadmium. Olafson and Thompson's (1974) report of increased quantities of cadmium binding protein with greater dose of cadmium suggests that fish are also capable of detoxifying greater amounts with increasing exposure. Such a mechanism might explain the flat mortality curve observed in this experiment.

Incipient lethal concentrations of cadmium for rainbow trout are considerably less than those reported for other types of fish. Pickering and Gast (1972) report a seven day TLm of 7.2 mg Cd/l using fathead minnows (Pimephales promelas). This threshold level as compared to 0.03 mg Cd/l in this study indicates that rainbow trout and likely salmonids in general will suffer toxic effects at much lower concentrations than will other types of fish.

The rate of cadmium accumulation in all tissues was greater at 18° than at 6°C. The rate of accumulation can be related to resting metabolic rate which doubles as the temperature of acclimation changes from 8 to 16°C (Evans et al. 1962). Tissue residues at mortality were comparable in 6 and 18° exposed and acclimated fish, but time to mortality of cold acclimated fish was approximately double that of warm acclimated fish.

Increases of accumulation rate at higher temperature were also reported to occur with rainbow trout exposed to mercury (MacLeod and Pessah 1973) and in the gill tissue of Atlantic salmon exposed to zinc (Hodson 1975). These investigators also reported increased survival time at lower temperatures. It appears that rate of accumulation and survival time are governed to a great extent by metabolic rate.

Cadmium generally accumulates to high levels in gill, liver and kidney of fish (Kumada et al. 1973, Benoit et al. 1976, Eaton 1974). It is therefore tempting to suppose that these organs are primary sites of cadmium's action, however, Nordberg (1974) indicates that cadmium in liver and kidney is largely bound to metallothionein- the cadmium binding protein. Liver and kidney may be involved to a great extent in sequestering cadmium and rendering it inactive. Kumada et al. (1973) report that rainbow trout exposed to cadmium (5ug/1) for thirty weeks retained high proportions in liver and kidney after transfer to clean water for ten weeks, but lost most of the cadmium associated with the gill. Similar results are reported by Benoit et al. (1976) with brook trout exposed to cadmium. Cadmium associated with gill tissue appears to be loosely bound and is lost quickly on transfer to clean water, whereas kidney and liver are primary sites of cadmium binding.

Sublethal effects

Growth is an essential biological function and it is often used as a measure of the suitability of an organism's environment. As such it is an important parameter to consider when investigating the influence of a toxic substance on fish.

Sprague (1971) suggests that the sublethal effects of a toxicant may be integrated as a change in growth, however, in order for the effects

to be manifested as a change in growth, significant changes in respiratory activity or interference with energy requiring processes must take place. Growth of rainbow trout is affected by sublethal concentrations of cyanide (Dixon 1975) and as an inhibitor of cytochrome oxidase (Leduc 1978), cyanide can be expected to have a pronounced effect on growth. Mount (1968) found that growth of fathead minnows was reduced at concentrations of copper that did not show histological damage. Copper at sublethal concentrations causes significant increases in oxygen consumption (O'Hara 1971) and this may indicate respiratory interference by copper that would explain the growth reductions reported by Mount.

The growth of rainbow trout appears to be reduced markedly at higher sublethal concentrations of cadmium and seven fish died at the highest cadmium concentration (17.5 µg/L) at 12°C. Although analysis of variance does not show a significant difference among treatments, the decline in growth may be obscured to some extent by the high variance of individual per cent wet weight gains. The decrease seemed to be the result of lethargy and reduced feeding activity that may be due to the hypercalcemia observed at the higher concentrations. Hypercalcemia in humans results in general depression of nervous function and patients often show signs of anorexia (Wintrobe et al. 1974, p.1944).

Water content of tissues was not significantly affected by exposure to cadmium but seems to increase with temperature. This effect may be partially due to the larger size attained by fish exposed to the warmer temperatures (Love 1970 p.89). The difference may also be due to temperature since Houston et al. (1968) demonstrated that the water content of cold-acclimated fish was greater than that of warm acclimated fish.

Calcium concentrations in the blood of the rainbow trout increased dramatically when the fish were exposed to increasing concentrations of cadmium. At 18°, fish exposed to 9.2 ug Cd/1 had calcium concentrations in the blood that were more than twice the control levels. The maximum calcium increase of 12°-exposed fish occurred at the highest exposure concentration of 17.5 ug Cd/1 and was 1.9 times the control level. The maximum effect in 6°-exposed fish was 1.6 times that of control levels. The increase in calcium concentrations of the blood was most pronounced at the highest temperature.

Magnesium concentrations of the blood were also significantly higher than in controls at some of the higher cadmium concentrations and the greatest increase was 1.3 times the control levels. The extremely high elevations of calcium in blood as compared to magnesium, sodium and potassium indicate a fairly specific effect of cadmium on calcium regulation.

Control concentrations of calcium and magnesium were lower in the blood of 6°-acclimated fish than in warm-acclimated fish. Murphy and Houston (1977) and Houston et al. (1968) also report lower concentrations of magnesium and calcium in cold-acclimated rainbow trout.

Calcium is a regulator of membrane permeability (Prosser 1973 p.99) and the high concentrations of calcium in the blood may have produced associated increases in blood sodium and magnesium. The hypercalcemia observed in rainbow trout as a result of the sublethal exposure can have a number of profound effects. Normal calcium concentrations in the plasma of rainbow trout are similar to those in human plasma and are close to the limit of solubility of calcium phosphate. Hypercalcemia in humans, particularly if associated with increases in blood phosphate,

results in precipitation of calcium phosphate in blood vessels, connective tissue, gastric mucosa and in the lumen of kidney tubules (Wintrobe et al. 1974 p. 1944). In addition hypercalcemia causes anorexia, neuromuscular disturbances, disturbed kidney function, lethargy and occasionally coma.

Bengtsson et al. (1975) report vertebral damage in minnows (Phoxinus phoxinus) after 70 days exposure to low levels of cadmium and Pascoe and Matthey (1977) report spinal deformations in three spined stickleback (Gasterosteus aculeatus) exposed to cadmium. Cadmium caused larval deformities in fathead minnows exposed to 57 ug Cd/l (Pickering and Gast 1972) and Eaton (1974) reports crippling of larval bluegills at 80 ug Cd/l. Disturbances in calcium regulation observed in this experiment may be related to the high incidence of spinal deformities in fish chronically exposed to cadmium and would have pronounced effects during periods of rapid spinal development of larvae and juveniles.

Calcium regulation in fish is poorly understood. Although calcitonin is a hypocalcemic agent in humans and is found in the ultimobranchial glands of fishes, a hypocalcemic function in fish has not been demonstrated for this hormone. In addition a hormone with the analogous hypercalcemic function of parathyroid hormone in mammals has not been found in fish (Pang 1973).

The corpuscles of Stannius associated with the kidney of ray-finned fish appears to produce a hypocalcemic substance which Pang et al. (1974) have named hypocalcin. Fenwick and So (1974) demonstrated a 17-fold increase of ^{45}Ca influx through the gills of stanniectomized eels resulting in pronounced hypercalcemia. Fenwick (1976) attributed the

hypercalcemia to the increased production of Ca^{2+} - ATPase isolated from the gill tissue of stanniectomized eels. He concludes that the quantity of Ca^{2+}-ATPase is maintained by the corpuscles of Stannius.

An enzyme with characteristics similar to that of eel gill ATPase has been isolated from the gills of rainbow trout by Ma et al. (1974). It is likely that this enzyme is also involved in branchial calcium transport and may be under the influence of the corpuscles of Stannius.

The pronounced hypercalcemia observed in rainbow trout after twenty days sublethal exposure to cadmium indicates either greater uptake of calcium through the gills or intestines or greater resorption from bone or by the kidney. Direct stimulation of uptake or resorption seems unlikely since cadmium has been shown to inhibit calcium transport by rat kidney mitochondria (Saris and Jarvisalo 1977) and to inhibit uptake of calcium from the rat intestine (Yuhas et al. 1978). It seems more probable that inhibition of hypocalcemic function such as that of the corpuscles of Stannius would cause such an effect.

Kidney tissue, with which the corpuscles of Stannius are associated, was a primary site of cadmium accumulation. The hypercalcemic response of rainbow trout in this experiment was similar in extent to that reported by Fenwick (1976) in stanniectomized eels and inhibition of the hypocalcemic function of the corpuscles of Stannius by cadmium may have caused the hypercalcemia observed in this experiment. This effect of cadmium would also explain the slight hypermagnesemia that was observed. Ma et al. (1974) indicate that magnesium competes for the active site of Ca^{2+} - ATPase. Increased Ca^{2+} - ATPase activity following stanniectomy would increase magnesium transport into the blood as well as calcium but to a lesser extent.

Cadmium accumulation rate in tissues of rainbow trout after the sublethal exposure period indicated a temperature related effect like that observed after acute exposure. Accumulation rate was greatest at 18°C and least at 6°C, with 12°-acclimated and exposed gill, liver, kidney and whole fish residues showing an intermediate rate. This pattern can be partially ascribed to the increase in standard metabolism of rainbow trout as the temperature increases (Dickson and Kramer 1971) which would influence the rate of cadmium uptake through the gill. Gill and kidney accumulated cadmium to similar extents and liver and whole body residues were comparatively low. The increased accumulation rate at higher temperatures and the more pronounced hypercalcemia observed at these temperatures indicates that cadmium enters important tissues more rapidly at higher temperatures and produces more deleterious effects.

Cadmium residues, particularly in gill and kidney tissue, are higher than control levels at all concentrations above 2 µg Cd/L. Concentrations above this level also produced hypercalcemia in the rainbow trout. The concentration of 2 µg Cd/L appears to be the maximum acceptable toxicant concentration (MATC) for twenty days of sublethal exposure at all three temperatures. Benoit et al. (1976) suggest a similar MATC of 1.7-3.4 µg Cd/L following a three generation study of brook trout exposed to cadmium.

Changes in blood ion concentrations during acute exposure

Calcium in the plasma of rainbow trout exposed to lethal concentrations of cadmium declined steadily during the acute exposure period under all temperature regimes. In all cases concentrations were below 3.0 meq/L at the end of the exposure period. The hypocalcemia was accompanied by pronounced increases in magnesium at the higher temperatures, that appeared

to stabilize before the end of the exposure period. Magnesium concentrations at 6° at first showed a decline but subsequently recovered to levels slightly higher than in control fish. These effects are quite different from the fairly specific increase in calcium observed after twenty days sublethal exposure.

Symptoms of acute and sublethal exposure were quite different. During acute exposure fish exhibited hypersensitivity and uncontrolled muscle activity whereas chronically exposed fish never showed these symptoms and instead became lethargic at the higher concentrations. The hypercalcemia observed following sublethal exposure may be a transitory response and longer exposure may have indicated a recovery. Fenwick (1976) indicates that hypercalcemia following stanniectomy in his eels was such a transitory phenomenon. The acute and sublethal responses may differ but both point to an effect of cadmium on calcium regulation.

The hypocalcemia observed during acute exposure can be expected to influence nerve and muscle function considerably since the maintenance of appropriate concentrations of calcium is essential for these activities. The symptoms of hypersensitivity, rhythmic muscle contractions and tetany are also common symptoms of hypocalcemia in humans (Wintrobe et al. 1974 p.1944). Similar symptoms of acute cadmium poisoning in fishes have been reported by a number of researchers (Cearley and Coleman 1974, Benoit et al. 1976, Pascoe and Matthey 1977).

The time of calcium reduction to 3.0 meq/l follows the pattern of survival time very closely under all temperature regimes. A correlation coefficient relating calcium reduction time and survival time is 0.98 and the t value of 9.23 (n=5) indicates that the two are correlated with

99% confidence. Calcium reduction time of fish acclimated to 12° and exposed at 6, 12 and 18° showed an inverse logarithmic temperature dependence as did survival time and both calcium reduction time and survival time showed a similar deviation from temperature dependence when the fish were acclimated and exposed at 18°C.

The symptoms of acute cadmium poisoning and the strong relationship between calcium reduction time and survival time indicate that hypocalcemia is the direct cause of mortality. The similarity of the symptoms of cadmium poisoning observed in this experiment with those reported by other researchers using a variety of fish species indicates that hypocalcemia may be a common response to acute cadmium exposure.

Impaired renal function is a common effect of acute and chronic cadmium exposure in mammals (Nordberg 1974). Renal tubular damage was also reported by Gardner and Yevich (1970) in killifish (Fundulus heteroclitus) after exposure to cadmium. Damage to renal tubules may result in decreased resorption of calcium leading to excessive loss of calcium and subsequent hypocalcemia, Saris and Jarvisalo (1977) indicate that cadmium is accumulated by rat kidney mitochondria through an active transport mechanism in the same way as calcium and that this mechanism accumulates cadmium from low concentrations. Cadmium also strongly inhibits the calcium transport system and this would result in lower resorptive capability of the kidney tubules. Brierley(1967) reports that some heavy metals cause swelling of heart mitochondria by inducing uptake of magnesium and potassium. Since swelling of proximal tubules is a manifestation of cadmium poisoning a similar uptake of magnesium may be related to the hypermagnesemia that accompanies hypocalcemia.

Hypocalcemia may also result from inhibition of calcium transport by the gill tissue. Saris and Jarvisalo (1977) have demonstrated inhibition of calcium transport by rat kidney mitochondria and Brierley (1967) indicates that cadmium inhibits active transport of calcium by heart mitochondria. Since cadmium in the water is likely to enter through the gill, it may exert a pronounced influence on branchial function and suppress calcium transport as it does in other tissues. The link between hypocalcemia and mortality implicates the gill and kidney as the primary organs affected by cadmium, and interference with the activity of either may produce the hypocalcemia that was observed.

Effect of acclimation and exposure temperature

Rainbow trout acclimated to 12° and exposed to a lethal concentration of cadmium at 18°C die more quickly than those acclimated and exposed at 18°. The difference in survival time suggests that warm acclimation results in a change in metabolism which increases the trout's resistance time.

The standard metabolic rate of cold-acclimated salmonids is generally higher than that of warm-acclimated individuals when both are exposed to the same temperature (Peterson and Anderson 1969, Brett 1971). A comparatively greater metabolic rate of the 12°-acclimated rainbow trout at 18° as compared to that of 18°-acclimated trout exposed at 18° might account partially for the relatively shorter survival time of the colder-acclimated fish.

The small difference in survival time between 12° and 18° acclimated and exposed fish compared to those acclimated and exposed at 6°, suggests that temperature compensation may account for the deviation from temperature dependence of the 18°-acclimated fish in response to cadmium exposure.

Fish often exhibit temperature compensation at higher temperatures. Roberts (1973) indicates very little change in routine or resting oxygen

consumption of pumpkinseed sunfish (Lepomis gibbosus) in the range of 12 to 20°C. Dickson and Kramer (1971) report only slight increases of standard metabolism of rainbow trout in the range of 15-20°C. The strong compensation of metabolic rate during acclimation to warm temperatures would account for the slight difference in survival time of rainbow trout acclimated to 12 and 18°C.

The small difference in survival time between 6° acclimated fish and those acclimated to 12° and exposed to cadmium at 6° indicates that cold acclimation results in very little change in survival time and would suggest that 6° and 12° acclimated fish are in similar metabolic states. However, the longer survival times that are characteristic of low temperatures may have resulted in significant acclimation of the 12° acclimated fish to the test temperature of 6°C.

The survival time of fish acclimated to 12° and exposed to cadmium at 6, 12 and 18° shows a logarithmic relationship with temperature that is characteristic of reactions governed by changes in kinetic energy. The Q_{10} relating rate of mortality (inverse of survival time) and temperature was 3.98. The Q_{10} of standard metabolism of Atlantic salmon acclimated to 6° is 2.1 and for those acclimated to 18° it is 3.0 (Peterson and Anderson 1969). If the Q_{10} of rainbow trout is similar to that of Atlantic salmon , the much higher Q_{10} in this experiment indicates that survival time is not purely a function of metabolic rate but that other factors are involved which magnify the effect of temperature compared to its effect on metabolic rate alone.

These temperature experiments indicate that rainbow trout can be expected to survive greater concentrations of cadmium for longer periods of time at colder temperatures. The increased susceptibility of rainbow

trout to cadmium when the temperature is increased suddenly indicates that rapid mortality would result if the fish were exposed to high concentrations of cadmium in an area of thermal discharge. The trout would be most resistant during winter conditions.

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