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**LA THÈSE A ÉTÉ  
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THE EFFECTS OF SUBLETHAL CYANIDE  
EXPOSURE ON EXOGENOUS YOLK IN  
ESTRADIOL-INDUCED IMMATURE  
RAINBOW TROUT, SALMO GAIRDNERI.

Daniel Gabriel Cyr

A Thesis  
in  
The Department  
of  
Biological Sciences

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

March 1984

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Abstract

THE EFFECTS OF SUBLETHAL CYANIDE  
EXPOSURE ON EXOGENOUS YOLK IN  
ESTRADIOL-INDUCED IMMATURE  
RAINBOW TROUT, SALMO GAIRDNERI.

Daniel Gabriel Cyr

Exogenous yolk production was induced in the liver of immature (25-35 g) rainbow trout, Salmo gairdneri, using two injections of estradiol (5 mg/kg of body weight) on days 0 and 3 of a seventeen day experimental period. Estradiol-induced rainbow trout were exposed to a sublethal concentration of 0.02 mg/L of HCN and a temperature of  $12.0 \pm 1.0^{\circ}\text{C}$ . Three series of experiments were performed with the following objectives: 1) to test the effects of HCN on the production of exogenous yolk; 2) to determine the effects of HCN on the release of yolk from liver; 3) to study the effects of cyanide pre-exposure on exogenous yolk production.

Estradiol-induced cyanide-exposed rainbow trout

exhibited significantly higher levels of exogenous yolk as compared to estradiol-induced fish on the twelfth day of a 17-day experiment; however, the amount of yolk present in the serum over the 17-day experiment was not altered by cyanide exposure. Estradiol-induced cyanide-exposed rainbow trout demonstrated rapidly rising mean serum calcium and serum phosphoprotein phosphorus levels which peaked on day 12 of a 17-day experimental period with levels of  $26.0 \pm 4.4$  mg% and  $277.0 \pm 69.8$  ug/ml (n=10) respectively. Mean serum levels in estradiol-induced rainbow trout increased throughout the experimental period to peak on day 17, with levels of  $24.2 \pm 7.4$  mg% and  $241.0 \pm 107.9$  ug/ml (n=9) respectively.

A seasonal difference was also observed in this study with respect to the effects of sublethal cyanide exposure on the circulating levels of exogenous yolk in the serum. Pre-exposure to cyanide, however, did not cause any significant effects on the inducibility of exogenous yolk production in rainbow trout.

Using immature male and female rainbow trout, a model was developed to test the effects of toxicants on exogenous yolk production in fish. The applications and implications of this model are also discussed.

## Acknowledgements

I would like to thank my supervisor, Dr. Sylvia M. Ruby, for her assistance and support as well as my research advisory committee, Dr. P.D. Anderson and Dr. G. Leduc, for their assistance throughout my studies. I would also like to thank Dr. David R. Idler (Director of Marine Sciences Research Laboratory, Memorial University of Newfoundland) for his vitellogenin analysis and for his sincere interest in this study.

I am ever grateful to my wife Mary for her uncompromising support and assistance as well as to my parents and family for their continual encouragement.

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## Introduction

The production of yolk in oviparous vertebrates, such as rainbow trout, occurs both endogenously, within the developing oocyte, and exogenously, outside the oocyte in the liver. Wallace and Jared (1968) demonstrated that exogenous yolk, as measured by circulating serum phosphoprotein phosphorus levels, in the female African clawed toad, Xenopus laevis, was synthesized in the liver and released into the serum following estradiol injections. Furthermore, these researchers demonstrated that male toads could be induced to produce exogenous yolk by injecting them with estradiol. This suggested that exogenous yolk production was not sex-linked but rather that circulating levels of estrogens were the responsible factors for controlling exogenous yolk synthesis. While they were able to induce exogenous yolk synthesis in the toads, they observed that the uptake of this exogenous yolk was not stimulated by estradiol. Further studies by Redshaw and Follett (1971) observed that that uptake of vitellogenin (exogenous yolk), by the developing oocyte, and its crystallization as a yolk platelet was stimulated by

gonadotropic hormones. A similar mechanism for exogenous yolk production in fish was first reported by Plack et al. (1971). In their study, female cod (Gadus morhua L.) were ovariectomized and injected with 1 mg/kg of body weight of B-estradiol. Exogenous yolk in the serum was identified using immunological techniques, in which antiserum to cod eggs was incubated with cod serum. The data were then quantified using immunoelectrophoresis and chromatography on Sephadex G-200 and TEAE-cellulose columns. The results indicated the presence of egg proteins in the serum of estradiol-induced male and female cod. These egg proteins consistently increased in concentration for 10 days, following an intramuscular estradiol injection of 1 mg/kg body weight.

In teleosts, vitellogenesis and oocyte maturation are under the control of the anterior pituitary gland (Peter, 1981). Upadhyay et al. (1978) demonstrated that while oocyte maturation in immature rainbow trout, Salmo gairdneri R., could be induced with injections of purified salmon gonadotropin, the incorporation of exogenous yolk could be accomplished

only when salmon pituitary extracts were injected three times per week for ten weeks. Their results suggested that while gonadotropin was necessary for oocyte maturation and exogenous yolk synthesis, some other hormone(s) appeared to be responsible for initiating yolk uptake by the developing oocyte. Ng and Idler (1979) demonstrated that there are two gonadotropins involved in the control of vitellogenesis and oocyte maturation in female chum salmon, Onchorynchus keta. The first of these gonadotropins (ConA-II GtH) is carbohydrate rich and is responsible for stimulating estrogen production by the ovarian follicle, as well as initiating oocyte maturation. The second gonadotropin (ConA-I GtH), which is low in carbohydrates, is responsible for initiating the pinocytotic uptake of exogenous yolk from the serum by the developing oocyte. From these experiments, it is possible to predict the hormonal control of yolk production in fish. The hypothalamus releases a gonadotropin-releasing hormone (GnRH), which stimulates the release of gonadotropins from the anterior pituitary into the serum. One of the gonadotropins (ConA-II GtH) initiates oocyte maturation and induces the ovarian follicles to

synthesize and release estrogens. The estrogens subsequently act upon the liver to stimulate exogenous yolk synthesis and release into the serum. The second gonadotropin acts upon the oocyte and stimulates the incorporation of exogenous yolk by the developing oocyte. The exogenous yolk is then converted to phosphovitin and lipovitellin and deposited as secondary yolk (Figure 1).

van Bohmen and Lambert (1981) have demonstrated that the production of exogenous yolk, or vitellogenin, in the liver of sexually mature female rainbow trout, Salmo gairdneri, occurs naturally each year from October to December, with peak serum levels occurring in December. The short period of time during which exogenous yolk is produced offers limited opportunity for studying the effects of toxicants on this important phase of fish reproduction. Whitehead et al. (1978) demonstrated that the production of exogenous yolk in sexually mature rainbow trout, Salmo gairdneri, is marked by an increased hepatosomatic index with elevated serum calcium and serum phosphoprotein phosphorus levels. While the production of exogenous yolk in sexually

**Figure 1:** The hormonal control of yolk production in teleosts. The hypothalamus releases a gonadotropin releasing hormone (GnRH) which stimulates the anterior pituitary to release gonadotropins (GtH). One of these gonadotropins then stimulates oocyte development as well as estrogen production by the ovarian follicles. One of these estrogens, estradiol, subsequently acts upon the liver to stimulate exogenous yolk production and release into the serum. This exogenous yolk is then sequestered from the serum by the developing oocyte following stimulation by a second gonadotropin. Finally the exogenous yolk, or vitellogenin, is converted to phosvitin and lipovitellin by the oocyte, and deposited as secondary yolk. Note that by injecting the fish with estradiol it is possible to overcome the control of the anterior pituitary.

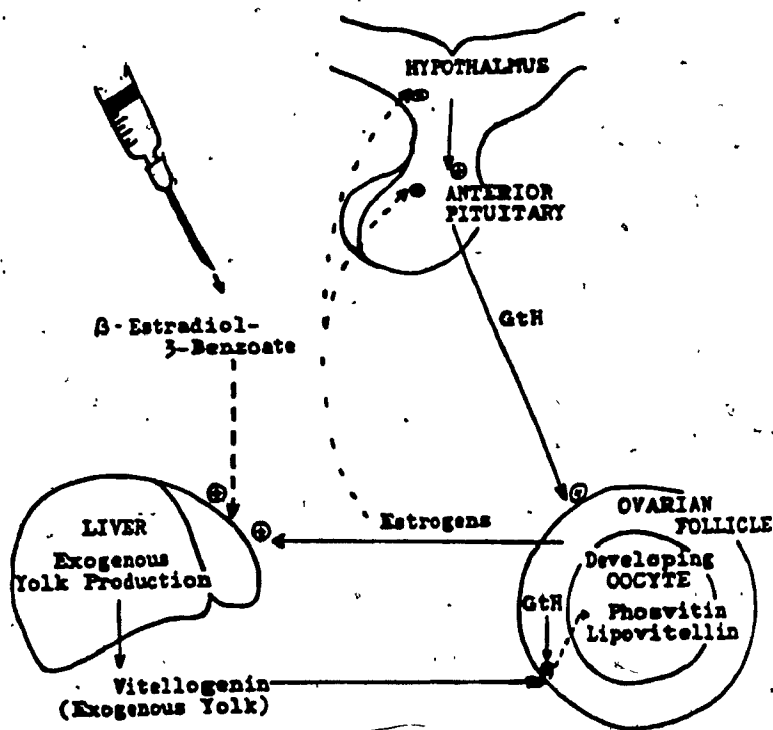


Figure 1

mature female fish is controlled by estrogens released from the ovary, Plack et al. (1971) have shown that the production of exogenous yolk can be induced in immature cod with intramuscular injections of estradiol. Campbell and Idler (1980) have also induced vitellogenin production in immature rainbow trout, Salmo gairdneri R., with intraperitoneal injections of estradiol. Both studies indicate that the capacity for exogenous yolk production is not sex-linked and can be induced in both male and female immature fish.

The use of cyanide in industrial processes, and its release into the ecosystem as part of industrial waste, has generated a growing concern regarding the sublethal effects of this toxicant upon fish reproduction. Ruby et al. (1979) found that the spermatogonia in testis of male rainbow trout, Salmo gairdneri, exposed for 18 days to sublethal cyanide (HCN) concentrations of 0.01 and 0.03 mg/L were unable to complete mitosis thereby decreasing the number of viable sperm by as much as 50 %. The effects of sublethal cyanide exposure on female reproduction in fish was first reported by Koenst et

al. (1977) who reported a reduction in the number of eggs spawned as well as a decrease in the viability of eggs in female brook trout, Salvelinus fontinalis, exposed for 144 days to 0.012 mg/L HCN. Similarly, Lind et al. (1977) also found a decrease in the number of eggs spawned in female fathead minnows exposed to 0.019 mg/L for 256 days. At a higher concentration of 0.05 mg/L HCN, Kimbal et al. (1978) demonstrated that fecundity was reduced to zero following a 289 day exposure in female bluegills, Lepomis macrochirus. Furthermore, Cheng and Ruby (1981) reported that american flagfish, Jordenella floridae, subjected to 5-day intermittent exposures to sublethal cyanide concentrations ranging from 0.065 to 0.15 mg/L during embryonic and juvenile development demonstrated a 40 % reduction in egg production at sexual maturity. In order to understand the mechanism by which sublethal cyanide exposure inhibited egg production in fish, Lesniak and Ruby (1982) conducted a histological study in which they exposed female rainbow trout, Salmo gairdneri, to 0.01 and 0.02 mg/L for 20 days. Their results demonstrated that female rainbow trout, exposed to 0.02 and 0.01 mg/L HCN exhibited an inhibition of



oocyte maturation as well as a significant reduction in yolk deposition in developing oocytes of the ovaries. While the results of the latter study dealt with egg yolk obtained from both endogenous and exogenous origin, there are no previous studies in the literature dealing specifically with the effects of sublethal cyanide exposure on exogenous yolk in fish.

The present study had two objectives. The first objective was to examine the effects of sublethal exposure to 0.02 mg/L cyanide on the production and release of exogenous yolk from the liver. The second objective was to develop a laboratory-controlled experimental model to test the effects of environmental toxicants upon exogenous yolk production and release in fish. Toxicological studies of this nature are normally difficult, due to the small proportion of females which undergo exogenous yolk production (i.e. vitellogenin) each year. A second limitation is the short period of time during which females actively produce exogenous yolk. The present model overcomes both of these limitations. First, it increases substantially the sample size for

each experiment, since both immature male and female trout can be induced. It also offers an opportunity to study exogenous yolk production throughout the year, and allows work with various classes of toxicants. Finally, it overcomes the difficulties inherent when holding large numbers of sexually mature fish under laboratory-controlled conditions, where aggression frequently results in injury to females.

## Materials and Methods

### Materials

Immature rainbow trout, Salmo gairdneri, weighing between 25 and 35 grams were purchased from La Pisciculture Mont Sutton, Mont Sutton, Quebec. The fish were transported to the laboratory in pond water which was oxygenated prior to departure from the hatchery. Upon arrival at the Sir George Williams Campus of Concordia University, the fish were released into holding tanks and acclimated to laboratory conditions for at least three weeks prior to any experiments. A few hours after their arrival in the lab, and during the acclimation period, they were fed Ewos Trout Chow (40% protein) ad libitum every day.

During the experiments, the fish were fed a ration of 2% of the total fish weight per day of Ewos Trout Chow (40% protein) and held in one hundred and eighty (180) litre white translucent polyethylene tanks (55 x 125 cm), with a water inflow rate of two

litres per minute and a fish density of 32 fish per tank. The water inflow rate provided a 99% replacement of tank water every 2.75 hours, as calculated by Sprague (1973). The laboratory water supply consisted of City of Montreal water, which was dechlorinated with an active charcoal filter. The dechlorinated water was delivered to a head tank, where it was aerated by a twelve centimeter air stone through which compressed air was continuously passed (Figure 2). The aerated water leaving the head tank was subsequently delivered to the experimental assembly using plastic polyvinyl chloride (PVC) piping. The flow of water entering the experimental assembly was regulated to each tank by a graduated flow metre (Manostat Corporation, New York). The light source originated from fluorescent neon lights placed directly above the experimental assembly. The experimental area was curtained off by a dark plastic to minimize any external disturbances.

Mariotte bottles, containing a stock concentration of the toxicant, were placed directly above the experimental tanks. Using 21g Intramedic polyethylene tubing, the toxicant was delivered from

**Figure 2:** Schematic diagram representing the experimental assembly used in this study. Incoming dechlorinated water was aerated in the head tank and subsequently delivered to the experimental tanks. The flow of the water entering the tanks was regulated to 2.0 L/min. by the graduate flow metre. The toxicant was delivered to the tank from mariotte bottles placed above the tanks.

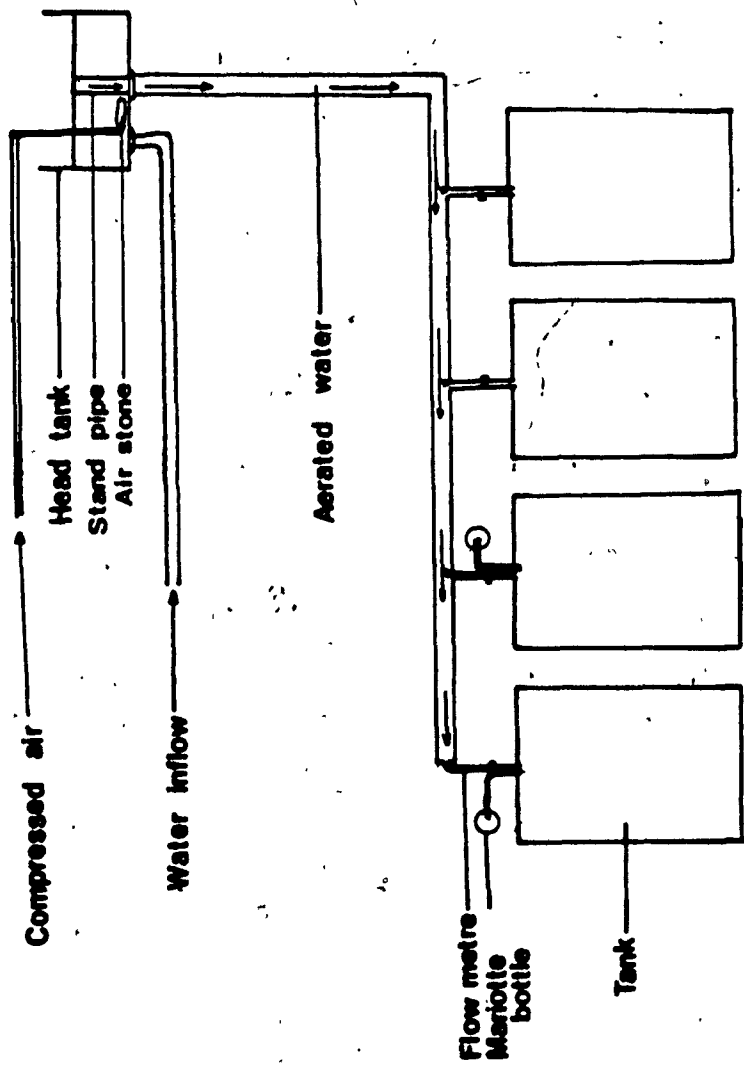


Figure 2

the Mariotte bottle to the tank according to the technique of Leduc (1966). The tubing was arranged along the side of the water inflow line and the toxicant mixed with inflowing water prior to entering the tank. This permitted the toxicant to enter the tank at the desired concentration. For each experiment, cyanide stock solutions were prepared by dissolving 20.0 g of NaCN (Baker Chemicals, Montreal) in 500 ml of distilled water. An appropriate volume of this stock solution was subsequently diluted in 18 litres of distilled water in a Mariotte bottle. Cyanide concentrations of 0.02 mg/L in the experimental tanks were monitored every two days following the method of Lambert et al. (1975). Cyanide concentrations in the experimental tanks did not vary by more than 5%.

The temperature of the water was regulated at  $12.0 \pm 1.0^{\circ}\text{C}$  and dissolved oxygen levels were maintained at  $95 \pm 2\%$ . Photoperiod was maintained on a twelve hour light, twelve hour dark regime which was controlled by an automatic timer.

## Methods

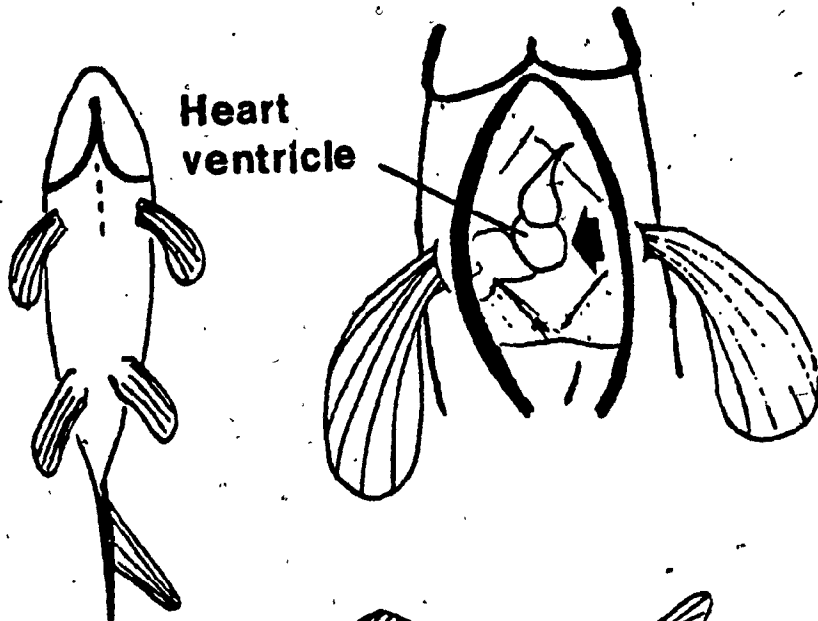
The experimental protocol utilized in all experiments for studying the effects of sublethal cyanide exposure on the production and release of exogenous yolk consisted of injecting immature rainbow trout with a dosage of 5 mg/kg of  $\beta$ -estradiol-3-benzoate on days 0 and 3 of a 17-day experimental period. Each experiment consisted of four experimental groups: a control, a cyanide-exposed, an estradiol-induced, and an estradiol-induced cyanide-exposed. Fish from each experimental group were sampled on days 1, 4, 7, 9, 12, and 17 of the experiment. Serum samples from each fish were subsequently analyzed for calcium and phosphoprotein phosphorus content and hepatosomatic indices were calculated for each fish. In the first series of experiments which investigated the effects of sublethal cyanide exposure on the production of exogenous yolk, cyanide exposure started on day 0. This resulted in the toxicant being introduced simultaneously with the induction of exogenous yolk production by estradiol. A second series of experiments determined the effects of sublethal cyanide exposure on the release of exogenous yolk



from the liver. In these experiments cyanide exposure started on day 4. The rationale for this is based on HSI values in estradiol-induced immature rainbow trout which indicate that most of the exogenous yolk had already been produced by the liver prior to its release into the serum. A third series of experiments, designed to study the effects of cyanide pre-exposure on the inducibility of exogenous yolk production, were conducted. Pre-exposure to cyanide began 13 days prior to the estradiol injections on days 14 and 17 of a 21-day experiment. In this experiment fish were sampled on day 21 in which peak HSI values had been observed in estradiol-induced immature rainbow trout. Experiments were also conducted to test the effects of the hormonal carrier peanut oil on the parameters being studied. In this experiment, immature rainbow trout were injected with peanut oil on days 0 and 3 of a twelve day experimental period. Fish were subsequently sampled on days 1, 4, 7, 9, and 12. Bleeding techniques were tested on 100-g rainbow trout. Each fish was bled by heart puncture and by removing blood from the dorsal aorta following caudal severing (Figure 3). Serum samples were subsequently analyzed for calcium and

Figure 3: Schematic representation of blood removed by heart puncture (A) and from the dorsal aorta by caudal severance (B). The dotted lines represent the area of incision while the arrows represent the location from which the blood was extracted (cross-section from Bond, 1979).

A



B

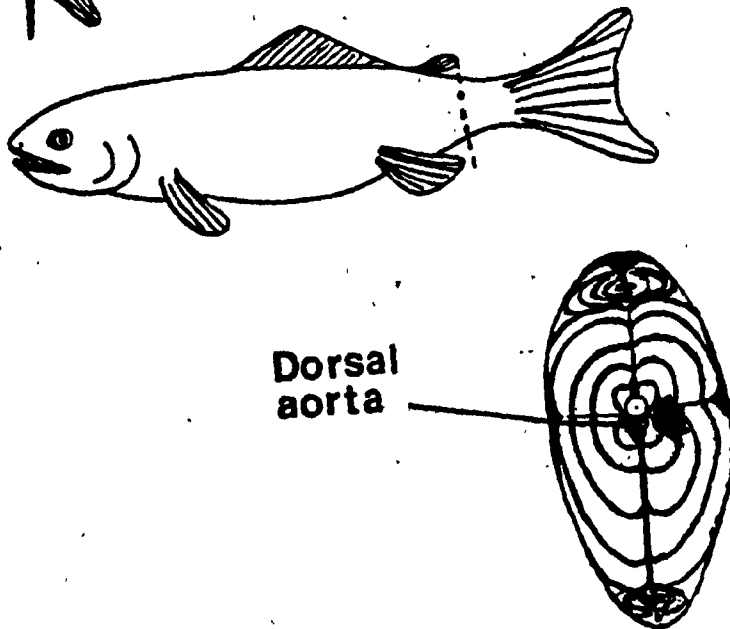
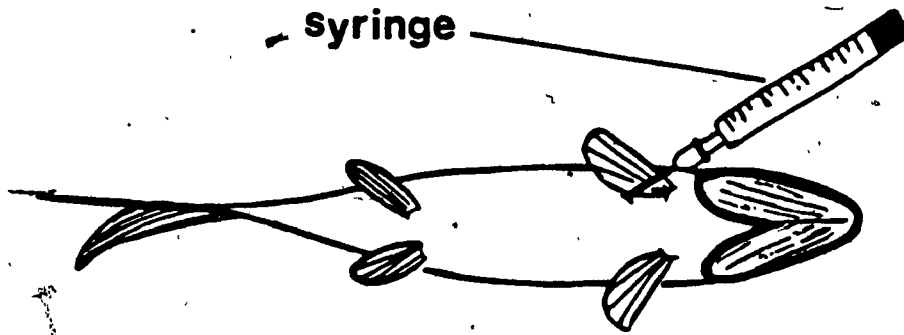


Figure 3

phosphoprotein phosphorus levels.

The preparation of the injected estradiol to induce ~~exogenous~~ yolk production consisted of dissolving  $\beta$ -estradiol-3-benzoate with constant stirring in 5.0 ml of peanut oil (Sigma Chemicals, St. Louis) for 16 hours prior to injection. At the time of estradiol injections, the fish were transported to the surgery room of the laboratory and held in an aerated water basin with a continuous water inflow. The procedure was performed under red fluorescent lighting to minimize stress. Individual fish were anaesthetized in water containing 0.4 g/L of 2-phenoxyethanol (Sigma Chemicals, St. Louis). They were then weighed and subsequently received intraperitoneal injections of 50  $\mu$ l of  $\beta$ -estradiol-3-benzoate at a dosage of 5 mg/kg of body weight (Sigma Chemicals, St. Louis). The injections were performed using a Hamilton 50  $\mu$ l glass syringe, equipped with a Yale 26g, 0.5-inch needle. Fish were held ventral side up and the needle was inserted at approximately 0.5 cm to the posterior of the right pectoral fin (Figure 4). The solution was slowly delivered and the needle gently removed to prevent

Figure 4: Schematic diagram representing the location where the needle was inserted at the time of estradiol injections. The injection was administered approximately 0.5 cm posterior to the right pectoral fin.



**Figure 4**

backflow of the injected estradiol from the peritoneal cavity. Following this, the fish were placed in a recovery tank containing well-aerated water for sixty to ninety minutes prior to being returned to the experimental tanks.

At the time of sampling, fish were killed with a sharp blow to the back of the head, and the tail was excised at the level of the caudal peduncle. Blood was collected from the dorsal aorta using a 1-cc Tuberculin syringe, previously rinsed in sodium citrate (25% w/v) to prevent blood clotting. The blood sample was emptied into a 1.5 ml Eppendorf micro test-tube and placed on ice. The samples were subsequently centrifuged for three minutes in a Brinkman Eppendorf Centrifuge at a force of 20,000 x g. This allowed the separation of the plasma from the red blood cells. The plasma was then allowed to clot and the resulting serum was then decanted from the micro test-tube and frozen at  $-70^{\circ}\text{C}$  in liquid nitrogen. The frozen samples were stored at  $-30^{\circ}\text{C}$  for future analysis of calcium and phosphoprotein phosphorus.

Serum samples were thawed prior to analysis, and separate serum samples of 50 ul each were used for calcium and phosphoprotein phosphorus analysis. The analysis of serum calcium consisted of diluting the serum samples to 5.0 ml with a solution of 2000 ppm. potassium chloride (KCl). Calcium content was determined by flame atomic absorption using a Perkin-Elmer Flame Atomic Absorption Spectrometer (Model 503), following the procedure outlined by Bhattacharya (1977). Serum levels were calculated from a standard curve derived from freshly prepared standards. Phosphoprotein phosphorus levels were obtained by precipitating the protein fraction of the serum with a 10% (w/v) solution of trichloroacetic acid (Sigma Chemicals, St. Louis). The solution was centrifuged for fifteen minutes in a Damon-IEC desk top clinical centrifuge (Model CL, #IM-174) at maximum speed. The trichloroacetic acid supernatant was discarded and the resultant pellet was delipidated according to the method of Wallace and Jared (1968). The protein pellet was then analyzed for phosphorus content using the procedures outlined in the Boehringer-Mannheim Test Handbook for Phosphorus and Phospholipids (1969).



Following the collection of blood samples, livers of individual fish were excised, the gall bladder removed, and liver weights were recorded. The hepatosomatic index (HSI) of individual fish was calculated by dividing the wet weight of the liver by the wet body weight of each fish and multiplying by 100.

$$\text{HSI} = \frac{\text{wet liver weight}}{\text{wet body weight}} \times 100$$

Statistical analysis of the data to test for significance between groups consisted of Student's T-Test (Apple, Statpak) for each sampling day. Areas under the curves produced over the seventeen day experimental periods were measured according to Simpson's Rule (Apple, Statpak) which is based on integrating the area under the curve. The areas under the curves were calculated for HSI, serum calcium, and serum phosphoprotein phosphorus levels for each experiment.

## Results

Preliminary experiments were performed to determine the optimal procedure for extracting blood from fish. This was based on previous reports which suggested that the procedures by which fish were bled could lead to differences in their total serum protein levels. To determine differences which might exist in serum calcium and phosphoprotein phosphorus levels between blood extracted from the dorsal aorta, by severing the tail at the level of the caudal peduncle, and heart puncture, 100 g rainbow trout (Salmo gairdneri) were bled using both techniques and serum subsequently analyzed. Mean serum calcium levels obtained by caudal severance were found not to be significantly different from the samples obtained by heart puncture with an identical mean value of  $8.6 \pm 0.7$  mg% obtained for both techniques (Figure 5). Serum samples obtained by caudal severance, demonstrated a higher mean serum phosphoprotein phosphorus level of  $40.9 \pm 21.8$   $\mu\text{g/ml}$  as compared to  $29.5 \pm 15.5$   $\mu\text{g/ml}$  ( $n=9$ ) for samples obtained by heart puncture (Table 1). While there were no significant ( $P < 0.05$ ) differences in serum phosphoprotein phosphorus

Figure 5: Histogram of mean values for serum calcium and serum phosphoprotein phosphorus levels in rainbow trout (100 g) from blood obtained by heart puncture and from the dorsal aorta, by caudal severance.

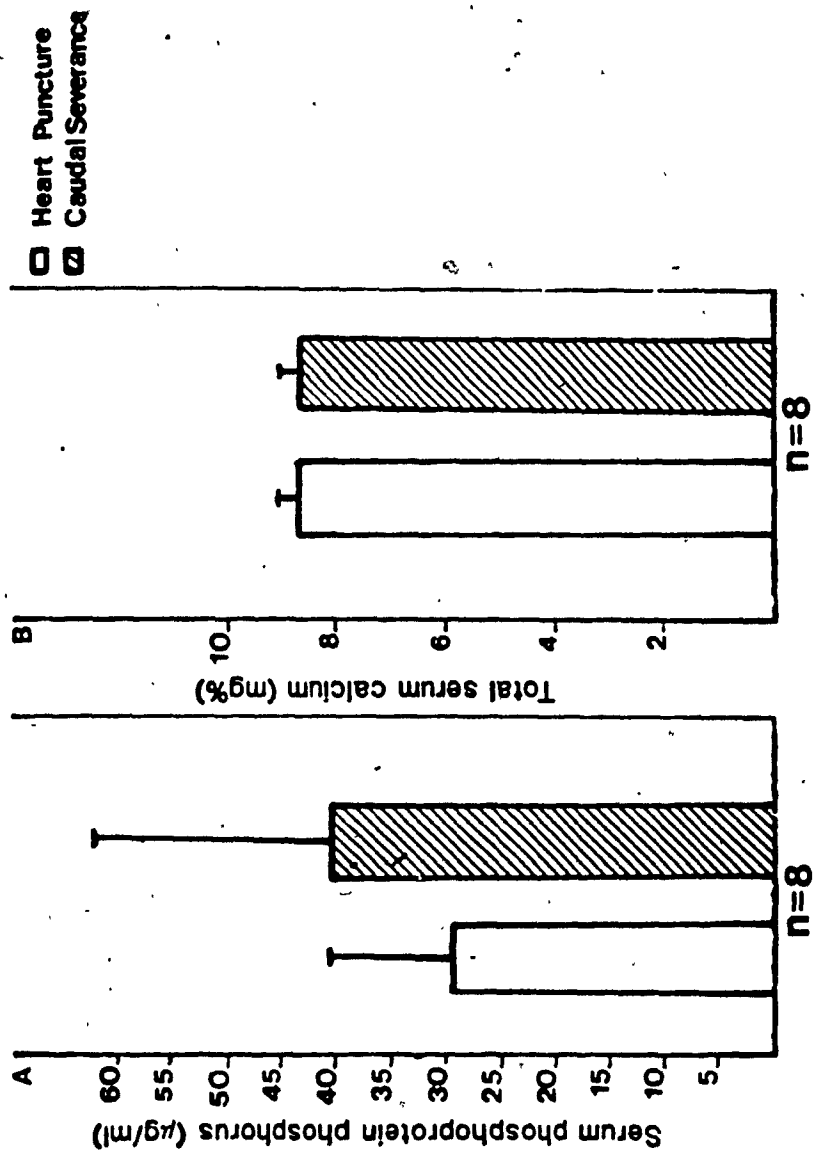


Figure 5

Table 1. Means and standard deviations in serum calcium and serum phosphoprotein phosphorus levels in rainbow trout bled by heart puncture and caudal severance techniques. The experiment was performed at  $12.0 \pm 1.0$  °C during February 1983. No significant differences ( $p < 0.05$ ) between techniques were observed for either parameter. Sample size = 8.

	Heart Puncture	Caudal Severance
Fish weight (g)	102.8 ± 30.5	102.8 ± 30.5
Serum Calcium (mg %)	8.6 ± 0.8	8.4 ± 0.7
Serum Phosphoprotein phosphorus (ug/ml)	29.5 ± 15.6	40.9 ± 21.8

level observed between both techniques, there was a trend towards higher serum levels in those samples taken by caudal severance (Figure 5). Larger 100 g rainbow trout were used in these experiments because of a greater blood volume, allowing sufficient blood to be removed for testing both caudal severance and heart puncture. In this study, the caudal severance technique was selected using smaller rainbow trout (25-35g) because of the high frequency of hemolysis (40%) and relatively small blood volume (50-100  $\mu$ l) obtained by heart puncture.

Based on previous reports in the literature, peanut oil was selected as the hormonal carrier for the estradiol injections. Preliminary experiments were also conducted to determine if this carrier caused any alterations on HSI values as well as on serum calcium and serum phosphoprotein phosphorus levels. Injections of peanut oil on days 0 and 3 of a twelve-day experimental period did not result in any significant differences ( $P < 0.05$ ) from untreated controls in HSI, serum calcium, and serum phosphoprotein phosphorus levels (Table 2, Figure 6).

Figure 6: Mean HSI, serum calcium, and serum phosphoprotein phosphorus levels in rainbow trout intraperitoneally injected with 50  $\mu$ l of peanut oil on days 0 and 3 of a twelve day experiment at  $12.0 \pm 1.0^\circ\text{C}$  during June 1983.

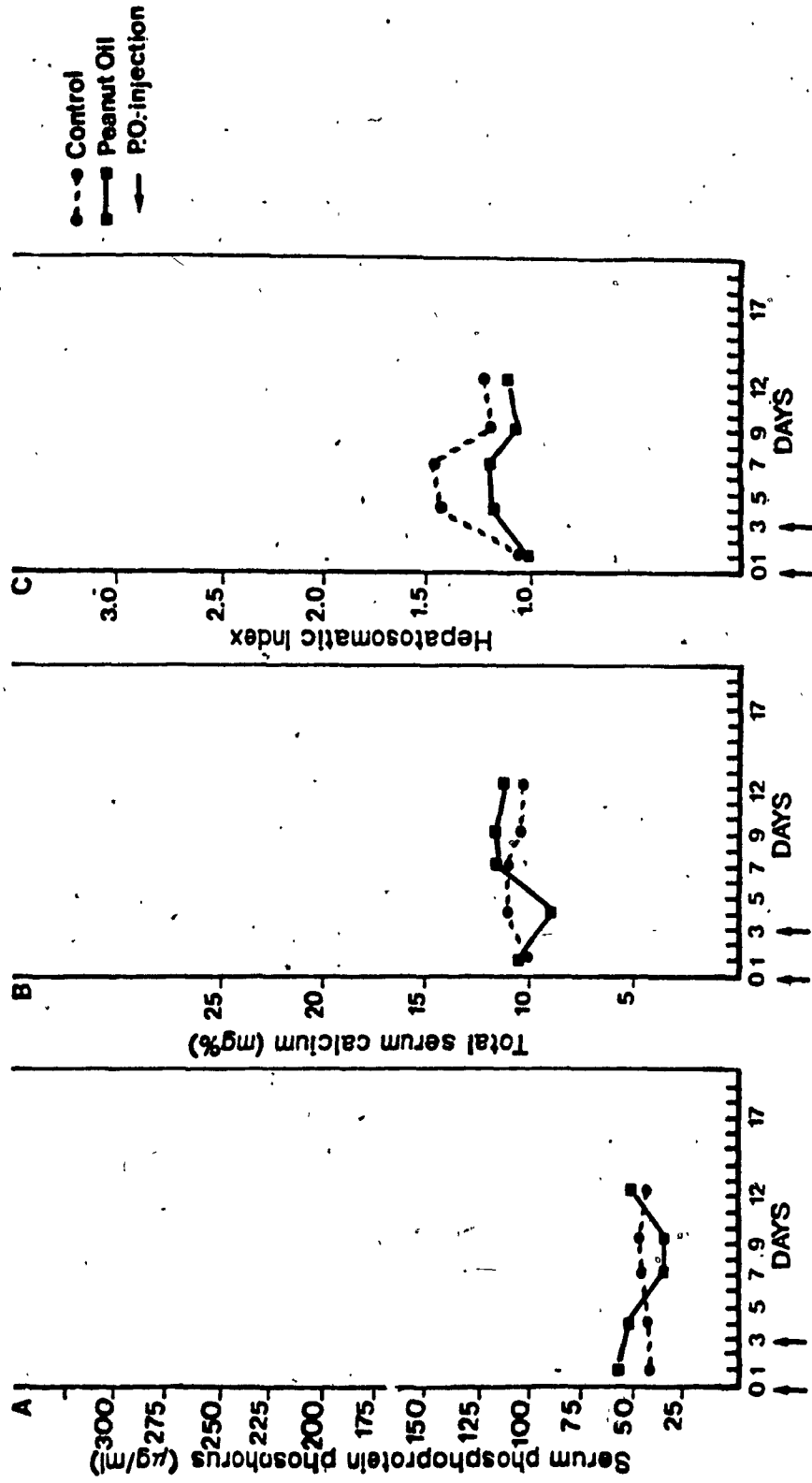


Figure 6



Table 2. Means and standard deviations of HSI, serum calcium, and serum phosphoprotein phosphorus levels in rainbow trout, intraperitoneally injected with 50 ul of peanut oil on days 0 and 3 of a 12-day experimental period, at  $12.0 \pm 1.0^\circ\text{C}$  during June 1983. No significant differences ( $p < 0.05$ ) between control and peanut oil injected fish were observed.

Day sampled	Group	HSI (%)	Serum calcium	Serum phosphoprotein phosphorus (ug/ml)
1	Control (n=5)	1.04 $\pm$ 0.17	10.1 $\pm$ 0.6	39.3 $\pm$ 24.9
	P. oil (n=5)	1.03 $\pm$ 0.11	10.8 $\pm$ 0.7	59.2 $\pm$ 20.8
4	Control (n=5)	1.44 $\pm$ 0.08	11.1 $\pm$ 1.6	40.9 $\pm$ 10.2
	P. oil (n=5)	1.20 $\pm$ 0.15	8.5 $\pm$ 1.2	53.7 $\pm$ 13.7
7	Control (n=5)	1.46 $\pm$ 0.26	11.3 $\pm$ 0.6	43.2 $\pm$ 22.9
	P. oil (n=5)	1.22 $\pm$ 0.07	11.5 $\pm$ 1.1	30.2 $\pm$ 8.9
9	Control (n=5)	1.20 $\pm$ 0.09	10.4 $\pm$ 0.5	45.8 $\pm$ 25.8
	P. oil (n=5)	1.06 $\pm$ 0.12	11.9 $\pm$ 1.0	30.0 $\pm$ 10.0
12	Control (n=5)	1.23 $\pm$ 0.04	10.2 $\pm$ 1.1	41.3 $\pm$ 13.7
	P. oil (n=4)	1.12 $\pm$ 0.07	11.3 $\pm$ 0.6	49.8 $\pm$ 10.5

For induction of exogenous yolk production in immature rainbow trout, estradiol injections were administered on days 0 and 3 of a seventeen-day experiment. This resulted in a significant increase ( $P > 0.01$ ) in HSI, which peaked on day 7, with a mean value of  $2.53 \pm 0.50\%$  ( $n=9$ ). Untreated controls displayed a mean HSI value of  $1.43 \pm 0.22\%$  ( $n=10$ ). On day 9, the mean HSI value in the estradiol-induced rainbow trout had decreased to  $2.14 \pm 0.29\%$  ( $n=10$ ) before again increasing to  $2.28 \pm 0.31\%$  ( $n=9$ ) by day 12. Mean HSI values in untreated controls decreased to  $1.16 \pm 0.14\%$  ( $n=10$ ) by day 9 and remained constant at  $1.17 \pm 0.11\%$  ( $n=10$ ). HSI values for estradiol-induced rainbow trout declined to an average value of  $1.42 \pm 0.25\%$  ( $n=9$ ) by day 17 while the average control value was  $1.19 \pm 0.15\%$  ( $n=9$ ) (Table 3). Both serum calcium and phosphoprotein phosphorus levels were not significantly different ( $P < 0.05$ ) between estradiol-induced and control rainbow trout until day 7. Serum calcium levels on day 7 were found to be slightly higher in estradiol-induced fish with a mean level of  $12.8 \pm 1.4\text{ mg\%}$  ( $n=9$ ) as compared to a mean level of  $11.5 \pm 3.8\text{ mg\%}$  in control fish while mean serum phosphoprotein phosphorus levels were significantly ( $P > 0.05$ ) higher in the estradiol-induced group with a mean

level of  $63.1 \pm 11.5$   $\mu\text{g/ml}$  ( $n=9$ ) while mean control levels were  $39.3 \pm 26.7$   $\mu\text{g/ml}$ . On days 9, 12, and 17, estradiol-induced rainbow trout displayed significantly ( $P \rightarrow 0.01$ ) higher mean levels of serum calcium and phosphoprotein phosphorus than the mean control values (Figure 7). Mean serum calcium levels in estradiol-induced rainbow trout were  $16.3 \pm 1.4$  mg% ( $n=10$ ),  $15.9 \pm 4.2$  mg% ( $n=9$ ), and  $24.2 \pm 7.4$  mg% ( $n=9$ ) for days 9, 12, and 17 respectively, while mean control levels were  $9.4 \pm 1.3$  mg% ( $n=10$ ),  $9.4 \pm 1.5$  mg% ( $n=10$ ), and  $10.9 \pm 1.6$  mg% ( $n=9$ ), respectively (Table 3). Mean serum phosphoprotein phosphorus levels in estradiol-induced rainbow trout over the same period of time displayed an identical pattern of change with a mean peak level of  $241.0 \pm 107.9$   $\mu\text{g/ml}$  ( $n=9$ ) on day 17. Mean serum phosphoprotein phosphorus level on day 17 in untreated control rainbow trout was  $58.9 \pm 24.2$   $\mu\text{g/ml}$  ( $n=9$ ) (Table 3).

The first series of experiments was designed to examine the effects of sublethal cyanide (0.02 mg/L) exposure on the production of exogenous yolk. In these experiments immature rainbow trout were injected with estradiol on days 0 and 3 of a 17-day experimental

Figure 7: Changes in mean HSI, serum calcium, and serum phosphoprotein phosphorus levels in rainbow trout, Salmo gairdneri, injected, intraperitoneally, with 5 mg/kg of estradiol on days 0 and 3 of a 17 day experiment. Fish were sampled on days 1, 4, 7, 9, 12, and 17. The experiment was conducted at  $12.0 \pm 1.0^{\circ}\text{C}$  and the graphs represent combined data from two experiments performed in January and March, 1983.

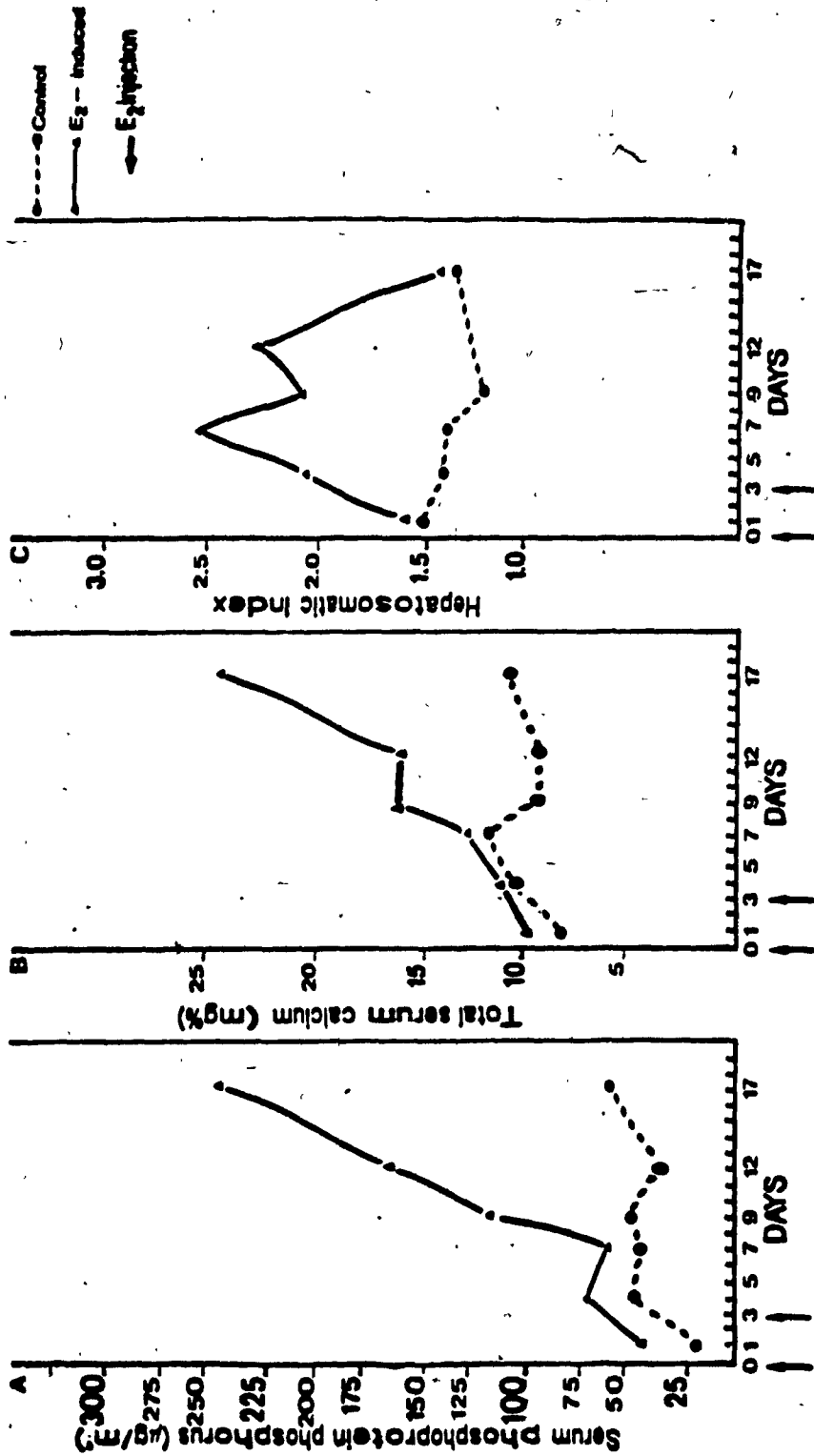


Figure 7

Table 3. Means and standard deviations of HSI, serum calcium, and serum phosphoprotein phosphorus, levels in rainbow trout following estradiol injections on days 0 and 3 of 17 day experiments. The experiments were performed at  $12.0 \pm 1.0^\circ\text{C}$  and the table contains the means of combined data of experiments conducted in January and March, 1983. Note: E2 = estradiol-induced; E2 + HCN = estradiol-induced, cyanide-exposed.

Day sampled	Group	HSI (%)	Serum calcium (mg%)	Serum phosphoprotein phosphorus (ug/ml)
1	Control (n=10)	$1.51 \pm 0.21$	$8.5 \pm 1.1$	$20.9 \pm 25.5$
	E2 (n=10)	$1.62 \pm 0.37$	$9.9 \pm 0.9$	$42.1 \pm 17.2^{\dagger\dagger}$
4	Control (n=10)	$1.40 \pm 0.25$	$10.8 \pm 2.2$	$45.4 \pm 35.3$
	E2 (n=10)	$2.08 \pm 0.26^{**}$	$10.3 \pm 1.0$	$71.5 \pm 65.1$
7	Control (n=10)	$1.38 \pm 0.28$	$11.5 \pm 3.8$	$39.3 \pm 26.7$
	E2 (n=9)	$2.53 \pm 0.05^{**}$	$12.8 \pm 1.4$	$63.1 \pm 11.5^{\dagger}$
9	Control (n=10)	$1.17 \pm 0.17$	$9.4 \pm 1.3$	$47.7 \pm 25.7$
	E2 (n=9)	$2.28 \pm 0.31^{**}$	$16.3 \pm 2.0^{**}$	$168.0 \pm 98.4^{**}$
12	Control (n=10)	$1.34 \pm 0.23$	$9.4 \pm 1.3$	$47.7 \pm 25.4$
	E2 (n=9)	$2.28 \pm 0.31^{**}$	$15.9 \pm 4.2^{**}$	$168.0 \pm 98.4^{**}$
17	Control (n=9)	$1.33 \pm 0.24$	$10.9 \pm 1.4$	$58.9 \pm 24.2$
	E2 (n=9)	$1.42 \pm 0.25$	$24.2 \pm 7.8^{**}$	$241.0 \pm 107.9^{**}$

\* P > 0.05; significantly different from controls.

\*\* P > 0.01; significantly different from controls.

† sample size = 9.

†† sample size = 6.

period in which exposure to cyanide started on day 0. For the experiment performed in June, 1983, no significant differences in HSI, serum calcium, and serum phosphoprotein phosphorus levels were observed between estradiol-induced and estradiol-induced cyanide-exposed rainbow trout (Figure 8). Mean HSI values for both groups peaked on day 7 with values of  $2.46 \pm 0.25\%$  (n=5) for estradiol-induced cyanide-exposed rainbow trout and  $2.30 \pm 0.07\%$  (n=5) for the estradiol-induced group. Serum calcium and phosphoprotein phosphorus levels in both groups increased throughout the experimental period, with mean peak levels on day 17 of  $32.4 \pm 7.3\text{ mg\%}$  (n=4) and  $274.0 \pm 95.1\text{ }\mu\text{g/ml}$  (n=5) respectively for estradiol-induced fish, and  $36.3 \pm 19.8\text{ mg\%}$  (n=4) and  $284.4 \pm 140.4\text{ }\mu\text{g/ml}$  (n=5) respectively for estradiol-induced cyanide-exposed rainbow trout (Table 4). Non-induced cyanide-exposed rainbow trout did not demonstrate any significant ( $P < 0.05$ ) differences in mean HSI, serum calcium, and serum phosphoprotein phosphorus levels from untreated control fish (Figure 9, Table 5).

The same experiment was repeated in October, 1983, but yielded different results (Figure 10). In this

Figure 8: Changes in mean HSI, serum calcium, and serum phosphoprotein phosphorus levels in rainbow trout, Salmo gairdneri, injected with 5 mg/kg of estradiol on days 0 and 3 of a 17 day experimental period in which exposure to a sublethal concentration of 0.02 mg/L of cyanide started on day 0. Fish were sampled on days 1, 4, 7, 12, and 17. The experiment was performed in June, 1983.



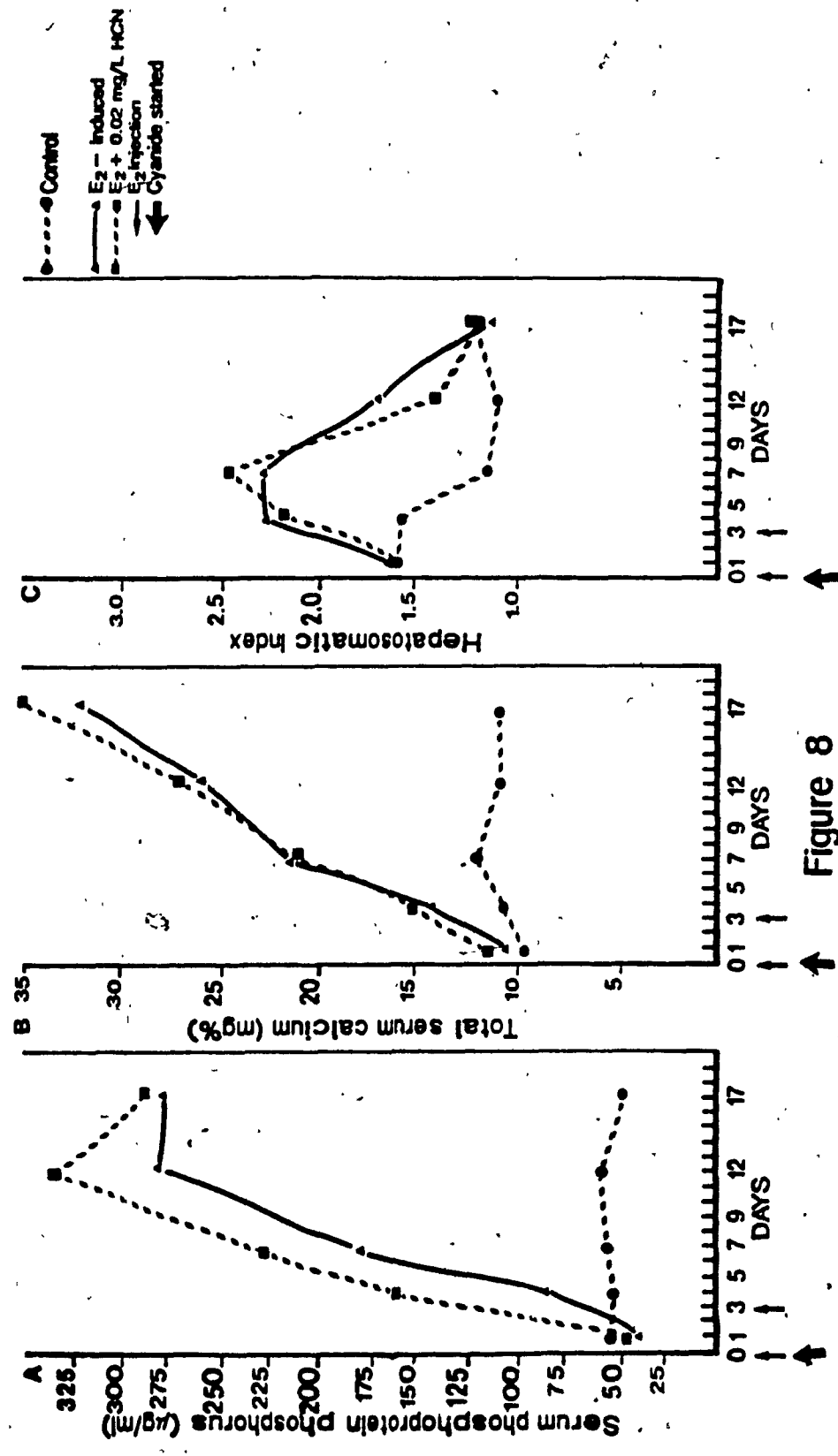


Figure 8

Table 4. Means and standard deviations in MSI, serum calcium, and serum phosphoprotein phosphorus levels in rainbow trout injected with 5 mg/kg of estradiol on days 0 and 3 of a 17-day experimental period in which exposure to 0.02 mg/L HCN started on day 0. Fish were sampled on days 1, 4, 7, 12, and 17. The experiment was performed at  $12.0 \pm 1.0^\circ\text{C}$  during June 1983. Note: E2 = estradiol-induced; E2 + HCN = estradiol-induced, cyanide-exposed.

Day sampled	Group	MSI (%)	Serum calcium (mg%)	Serum phosphoprotein phosphorus ( $\mu\text{g}/\text{ml}$ )
1	Control (n=5)	$1.60 \pm 0.20$	$10.8 \pm 0.6^\dagger$	$57.4 \pm 11.0$
	E2 (n=5)	$1.64 \pm 0.17$	$9.5 \pm 0.7$	$37.0 \pm 17.6$
	E2+HCN (n=5)	$1.61 \pm 0.12$	$11.9 \pm 1.8$	$46.4 \pm 7.6$
4	Control (n=5)	$1.57 \pm 0.14$	$11.0 \pm 1.4$	$57.6 \pm 31.6^\dagger$
	E2 (n=5)	$2.26 \pm 0.27^*$	$14.3 \pm 1.8^*$	$85.9 \pm 9.9$
	E2+HCN (n=5)	$2.15 \pm 0.15^*$	$15.1 \pm 0.5^{*†}$	$167.4 \pm 62.1^*$
7	Control (n=5)	$1.18 \pm 0.07$	$12.2 \pm 1.1$	$57.4 \pm 15.4$
	E2 (n=5)	$2.30 \pm 0.07^{**}$	$21.3 \pm 1.7^{**}$	$181.6 \pm 22.4^{**}$
	E2+HCN (n=5)	$2.46 \pm 0.25^{**}$	$21.0 \pm 2.8^{**†}$	$228.9 \pm 48.3^{**}$
12	Control (n=5)	$1.12 \pm 0.10$	$11.0 \pm 1.5$	$59.4 \pm 15.8$
	E2 (n=5)	$1.74 \pm 0.32^*$	$26.4 \pm 9.2^{**}$	$333.4 \pm 137.4^{**}$
	E2+HCN (n=5)	$1.37 \pm 0.27$	$27.5 \pm 7.3^{**}$	$278.6 \pm 99.8^{**}$
17	Control (n=4)	$1.26 \pm 0.18$	$11.2 \pm 0.8$	$49.0 \pm 17.9$
	E2 (n=5)	$1.17 \pm 0.12$	$32.4 \pm 7.3^{**†}$	$274.0 \pm 95.1^{**}$
	E2+HCN (n=5)	$1.24 \pm 0.17$	$36.3 \pm 19.8^{**†}$	$284.4 \pm 140.4^{**}$

\*  $p > 0.05$ ; significantly different from controls.

\*\*  $p > 0.01$ ; significantly different from controls.

† sample size = 4.

No significant differences ( $p < 0.05$ ) between E2-induced, and E2-induced cyanide-exposed fish groups.

Figure 9: Mean HSI, serum calcium, and serum phosphoprotein phosphorus levels in rainbow trout, Salmo gairdneri, ranging from 25 to 35 g, following exposure to 0.02 mg/L HCN for 17 days at  $12.0 \pm 1.0^\circ\text{C}$ , during June, 1983.

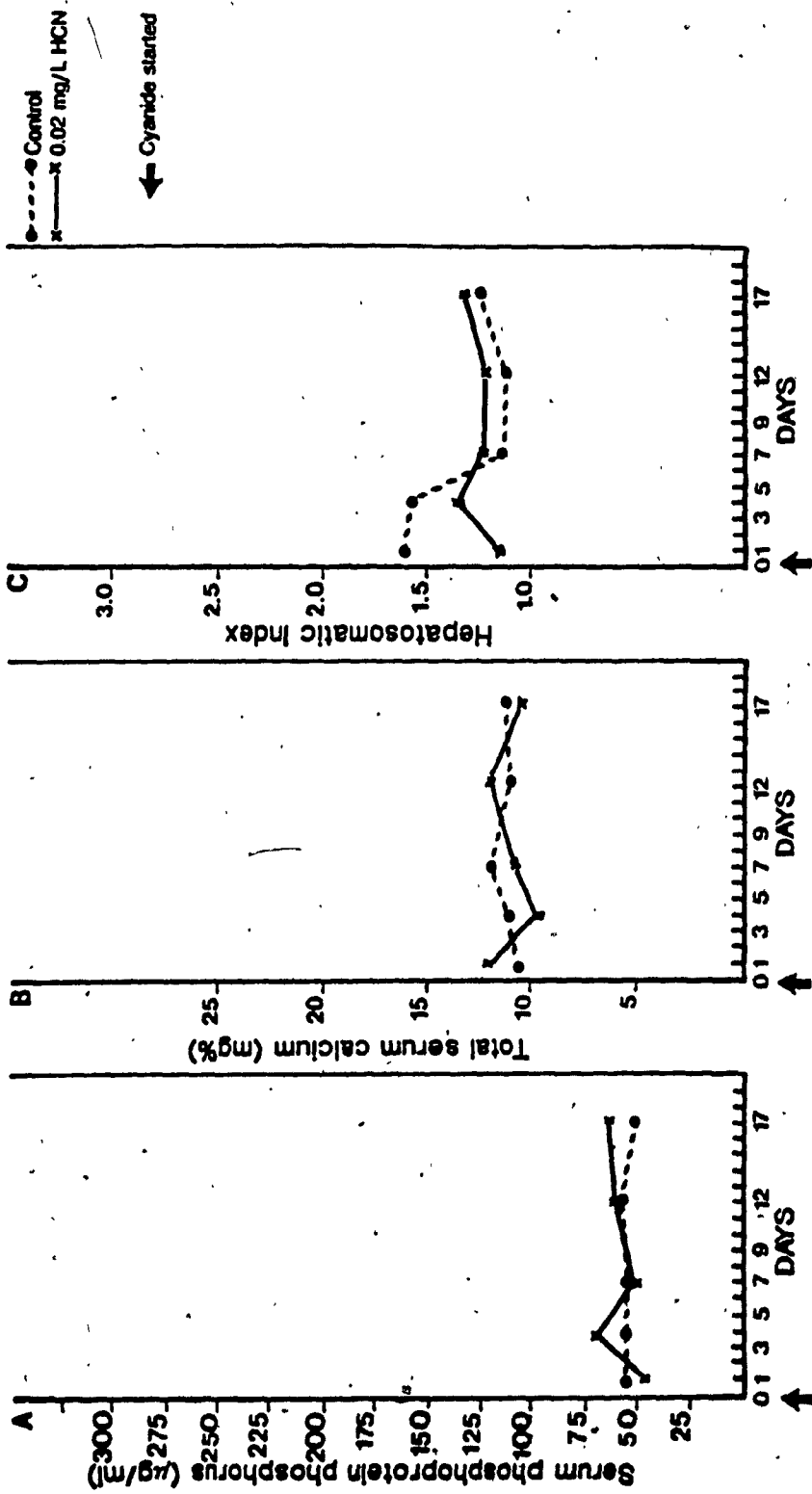


Figure 9

Table 5. Means and standard deviations of HSI, serum calcium, and serum phosphoprotein phosphorus levels in rainbow trout. Fish were exposed to 0.02 mg/L HCN from day 0 to 17 of the 17-day experimental period. The experiment was performed at  $12.0 \pm 1.0^\circ\text{C}$  during June, 1983. No significant ( $P < 0.05$ ) differences were observed between control and cyanide-exposed fish.

Day Sampled	Group	HSI (%)	Serum Calcium (mg%)	Serum Phosphoprotein Phosphorus ( $\mu\text{g/ml}$ )
1	Control (n=5)	$1.60 \pm 0.20$	$10.8 \pm 0.6$	$57.6 \pm 11.0$
	Cyanide (n=5)	$1.19 \pm 0.09$	$11.9 \pm 1.3$	$45.0 \pm 12.2$
4	Control (n=5)	$1.57 \pm 0.14$	$11.0 \pm 1.4$	$56.9 \pm 31.6$
	Cyanide (n=5)	$1.37 \pm 0.14$	$10.7 \pm 3.0$	$70.9 \pm 13.5$
7	Control (n=5)	$1.18 \pm 0.07$	$12.2 \pm 1.1$	$57.4 \pm 15.4$
	Cyanide (n=5)	$1.26 \pm 0.17$	$10.6 \pm 1.5$	$55.7 \pm 22.1$
12	Control (n=5)	$1.12 \pm 0.10$	$11.0 \pm 1.5$	$59.4 \pm 15.8$
	Cyanide (n=5)	$1.22 \pm 0.06$	$12.0 \pm 1.9$	$60.8 \pm 14.4$
17	Control (n=4)	$1.26 \pm 0.18$	$11.2 \pm 0.8$	$49.0 \pm 17.9$
	Cyanide (n=4)	$1.34 \pm 0.10$	$10.3 \pm 0.5$	$60.9 \pm 8.4$

experiment the results observed were similar to those observed in experiments in which cyanide exposure started on day 4. Serum calcium and phosphoprotein phosphorus levels began to increase by the seventh day of the experiment. Serum levels in estradiol-induced cyanide-exposed rainbow trout peaked on day 12, with significantly ( $P > 0.05$ ) higher mean serum calcium and phosphoprotein phosphorus levels of  $25.2 \pm 2.6$  mg% ( $n=6$ ) and  $367.4 \pm 43.3$   $\mu\text{g/ml}$  ( $n=5$ ) respectively as compared to mean levels of  $20.4 \pm 2.9$  mg% ( $n=3$ ) and  $213.5 \pm 4.5$   $\mu\text{g/ml}$  ( $n=3$ ) respectively, for estradiol-induced rainbow trout (Table 6). On day 17, mean serum calcium and phosphoprotein phosphorus levels in estradiol-induced cyanide-exposed rainbow trout declined to  $23.6 \pm 1.1$  mg% ( $n=5$ ) and  $213.5 \pm 4.5$   $\mu\text{g/ml}$  ( $n=5$ ) respectively, while mean levels in the estradiol-induced group had increased to  $22.8 \pm 2.8$  mg% ( $n=4$ ) and  $269.2 \pm 21.0$   $\mu\text{g/ml}$  ( $n=4$ ) respectively (Table 6). The areas under the curves for estradiol-induced and estradiol-induced cyanide-exposed rainbow trout were also calculated (Simpson's Rule, Apple Statpak, 1981). It was observed that the mean HSI values over the seventeen-day experimental period for estradiol-induced cyanide-exposed rainbow trout was 10.8 % lower than that of the estradiol-induced fish. Mean

Figure 10: Changes in mean HSI, serum calcium, and serum phosphoprotein phosphorus levels in rainbow trout, Salmo gairdneri, injected with estradiol on days 0 and 3 of a 17 day experimental period in which exposure to a sublethal concentration of 0.02 mg/L HCN started on day 0. Fish were sampled on days 0, 7, 12, and 17. The experiment was performed in October, 1983.

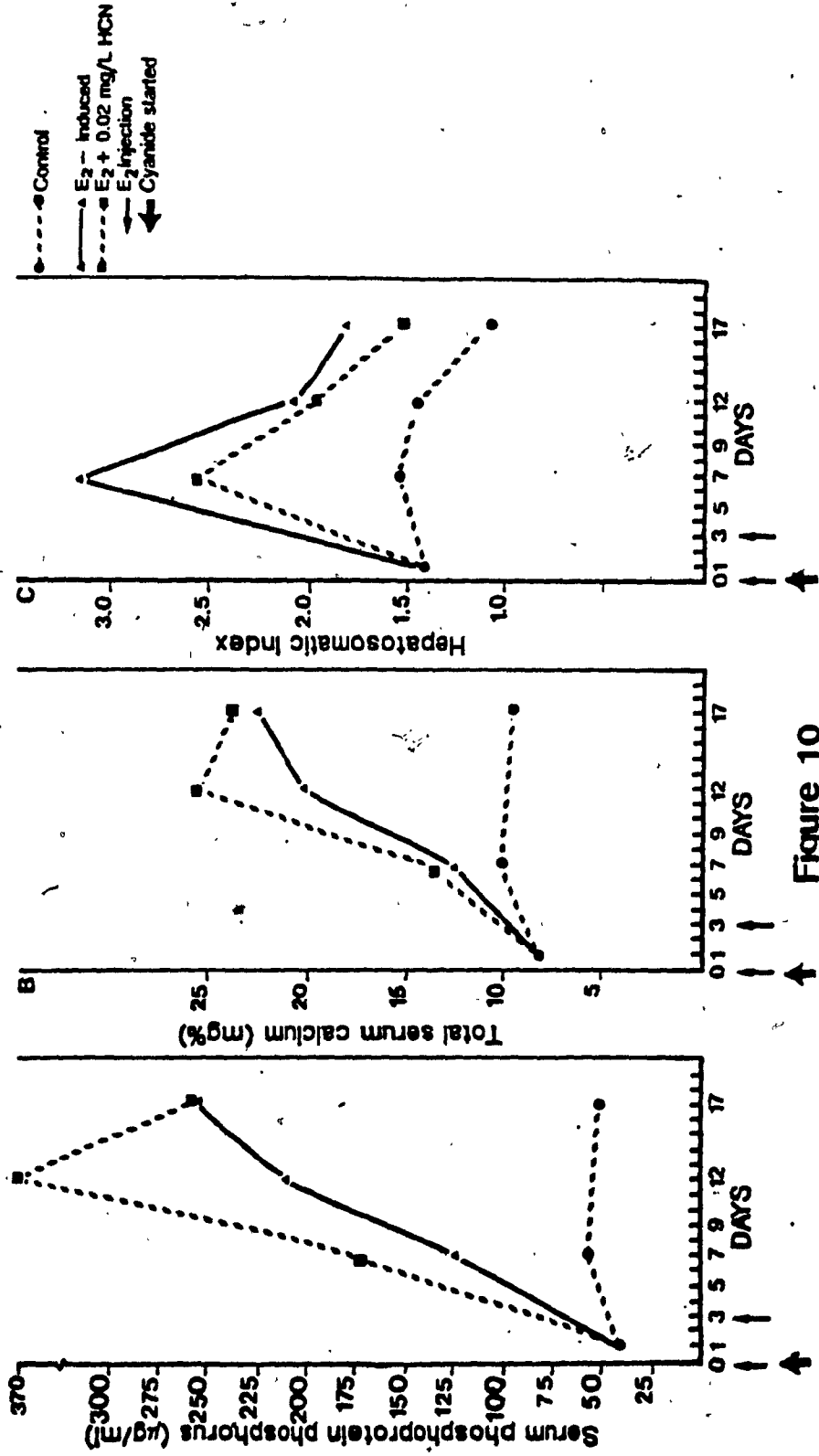


Figure 10



Table 6. Means and standard deviations in HSI, serum calcium, and serum phosphoprotein phosphorus levels in rainbow trout injected with 5 mg/kg of estradiol on days 0 and 3 of a 17-day experimental period in which exposure to 0.02 mg/L HCN started on day 0. Fish were sampled on days 1, 7, 12, and 17. The experiment was performed at  $12.0 \pm 1.0^\circ\text{C}$  during October, 1983. Note: E2 = estradiol-induced; E2 + HCN = estradiol-induced, cyanide-exposed.

Day sampled	Group	HSI (%)	Serum calcium (mg%)	Serum phosphoprotein phosphorus (ug/ml)
1	Control (n=5)	1.60 ± 0.20	10.8 ± 0.6	57.6 ± 11.0
7	Control (n=4)	1.52 ± 0.20	10.0 ± 0.9†	57.5 ± 19.4
	E2 (n=5)	3.18 ± 0.19**	12.6 ± 1.0*	122.6 ± 3.6*
	E2+HCN (n=5)	2.52 ± 0.05**	13.9 ± 0.5*	173.9 ± 27.6*
12	Control (n=4)	1.44 ± 0.17	-	-
	E2 (n=4)	2.06 ± 0.13**	20.4 ± 2.9†	213.5 ± 4.5
	E2+HCN (n=6)	1.93 ± 0.11**	25.2 ± 2.6	367.4 ± 43.3†
17	Control (n=4)	1.08 ± 0.03	9.3 ± 1.3	49.1 ± 9.4
	E2 (n=6)	1.81 ± 0.19*	22.8 ± 2.8**	269.2 ± 21.0**†
	E2+HCN (n=6)	1.52 ± 0.28	23.6 ± 1.1**	258.6 ± 24.5**†

\* p > 0.05; significantly different from controls.

\*\* p > 0.01; significantly different from controls.

† sample size = 3.

\*\* sample size = 3.

No significant differences (p < 0.05) between E2-induced and E2-induced cyanide-exposed fish groups.

serum calcium levels were 18.2 % higher in estradiol-induced cyanide-exposed rainbow trout, while the area for mean serum phosphoprotein phosphorus levels was 52.6 % greater in estradiol-induced cyanide-exposed fish as compared to estradiol-induced rainbow trout.

A second series of experiments were performed to examine the effects of sublethal cyanide (0.02 mg/L) exposure on the final stages of exogenous yolk production in the liver and its subsequent release into the serum. Following the estradiol injections on days 0 and 3 of a seventeen-day experimental period, the immature rainbow trout were exposed to a sublethal concentration of 0.02 mg/L of cyanide from day 4 of the experiment through to day 17. On the ninth day of the experiment, the mean HSI value of estradiol-induced cyanide-exposed rainbow trout was significantly ( $P > 0.05$ ) lower than that of the estradiol-induced group (Figure 11). The mean HSI value for estradiol-induced cyanide-exposed fish was  $1.72 \pm 0.31$  % ( $n=9$ ) as compared to  $2.14 \pm 0.29$  % ( $n=10$ ) for the estradiol-induced group. Mean HSI values in the estradiol-induced cyanide-exposed group continued to be significantly lower ( $P > 0.05$ )

Figure 11: Changes in mean HSI, serum calcium, and serum phosphoprotein phosphorus levels in male and female rainbow trout, Salmo gairdneri, following estradiol-injections on days 0 and 3 of a 17 day experiment in which cyanide (0.02 mg/L) exposure started on day 4. Fish were sampled on days 1, 4, 7, 9, 12, and 17. Experiments were performed at  $12 \pm 1.0^{\circ}\text{C}$  and the graphs represent combined data of two experiments performed in January and March, 1983.

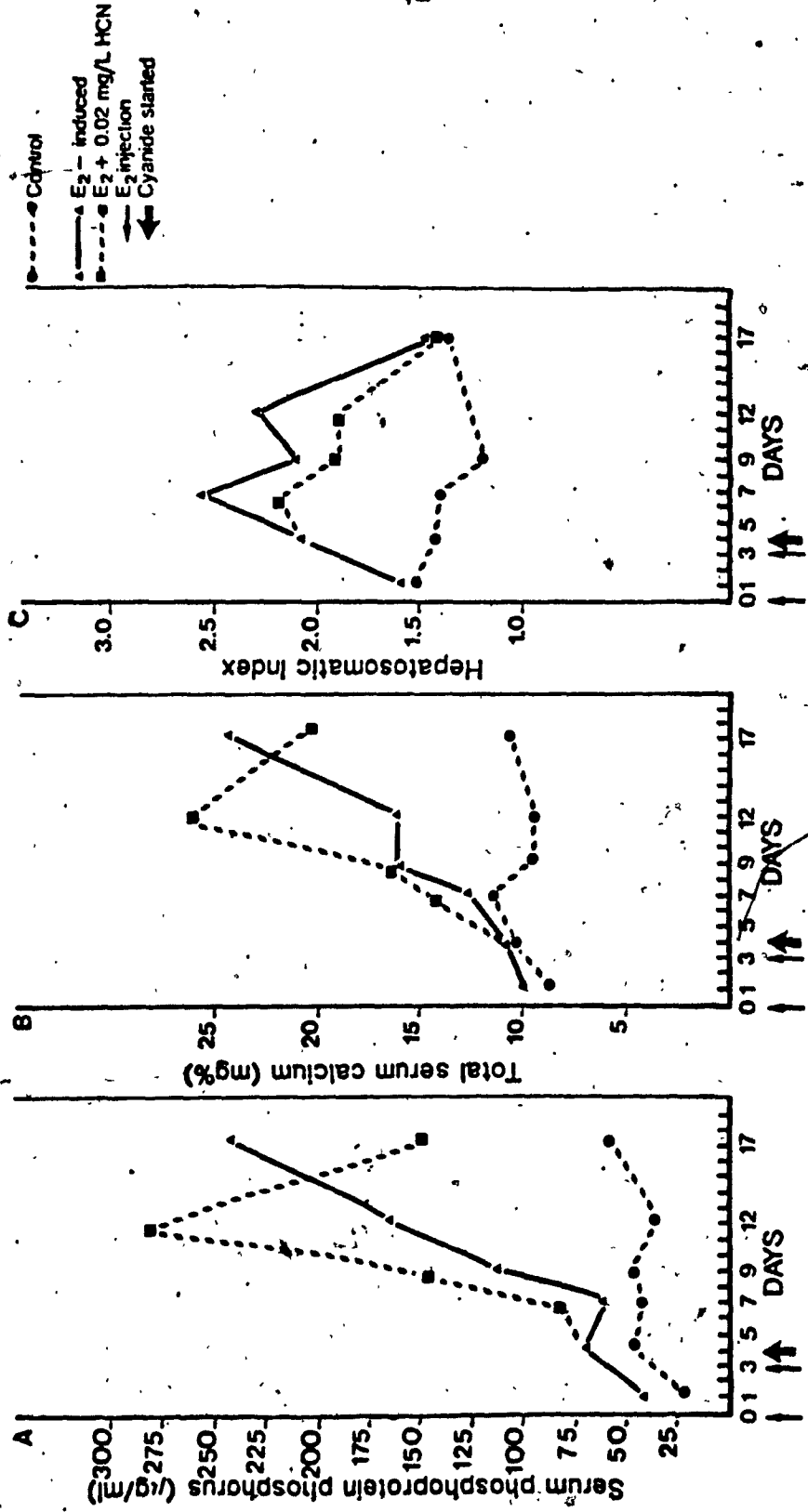


Figure 11

Table 7. Means and standard deviations of HSI, serum calcium, and serum phosphoprotein phosphorus levels in rainbow trout following estradiol injections on days 0 and 3 of a 17-day experiment in which exposure to 0.02 mg/L HCN started on day 4. Experiments were performed at  $12.0 \pm 1.0^\circ\text{C}$  during January, 1983.

Day sampled	Group	HSI (%)	Serum calcium (mg%)	Serum phosphoprotein phosphorus (ug/ml)
1	Control (n=5)	1.40 $\pm$ 0.24	8.1 $\pm$ 0.5	40.0 $\pm$ 27.7
	E2 (n=5)	1.97 $\pm$ 0.12**	9.8 $\pm$ 0.6	57.6 $\pm$ 30.6
4	Control (n=5)	1.49 $\pm$ 0.26	9.1 $\pm$ 0.8	76.4 $\pm$ 16.4
	E2 (n=5)	2.73 $\pm$ 0.29**	11.9 $\pm$ 1.8†	54.6 $\pm$ 14.0
7	Control (n=5)	1.57 $\pm$ 0.11	9.6 $\pm$ 0.8	57.2 $\pm$ 14.4
	E2 (n=5)	2.73 $\pm$ 0.29**	11.9 $\pm$ 1.8	54.6 $\pm$ 14.0
	E2+HCN (n=5)	2.43 $\pm$ 0.09**	12.1 $\pm$ 1.8*	97.4 $\pm$ 52.2
9	Control (n=5)	1.16 $\pm$ 0.08	8.8 $\pm$ 0.7	52.0 $\pm$ 6.9
	E2 (n=5)	2.40 $\pm$ 0.21**	14.5 $\pm$ 1.5**	121.5 $\pm$ 42.0*
	E2 + HCN (n=5)	2.09 $\pm$ 0.17**	13.8 $\pm$ 1.7**	143.1 $\pm$ 22.7**
12	Control (n=5)	1.21 $\pm$ 0.09	8.3 $\pm$ 0.8	30.7 $\pm$ 13.3
	E2 (n=4)	2.46 $\pm$ 0.20**	17.2 $\pm$ 2.8**	188.5 $\pm$ 51.9**
	E2+HCN (n=5)	2.09 $\pm$ 0.17	25.7 $\pm$ 1.7**	234.5 $\pm$ 51.3**
17	Control (n=4)	1.13 $\pm$ 0.11	10.2 $\pm$ 0.9	50.1 $\pm$ 9.5
	E2 (n=4)	1.48 $\pm$ 0.10	20.3 $\pm$ 2.0**	163.0 $\pm$ 28.2**
	E2+HCN (n=5)	1.56 $\pm$ 0.22	18.1 $\pm$ 4.4**	145.2 $\pm$ 44.2**

\* P > 0.05; significantly different from controls.

\*\* P > 0.01; significantly different from controls.

† sample size = 4.

Table 8. Means and standard deviations of HSI, serum calcium, and serum phosphoprotein phosphorus levels in rainbow trout following estradiol injections on days 0 and 3 of a 17-day experiment in which exposure to 0.02 mg/L HCN started on day 4. Experiments were performed at  $12.0 \pm 1.0^\circ\text{C}$  during March, 1983.

Day sampled	Group	HSI (%)	Serum calcium (mg%)	Serum phosphoprotein phosphorus ( $\mu\text{g/ml}$ )
1	Control (n=5)	$1.43 \pm 0.10$	$8.9 \pm 0.8$	$14.0 \pm 2.0$
	E2 (n=5)	$1.27 \pm 0.10$	$10.0 \pm 1.5$	$11.7 \pm 1.0$
4	Control (n=5)	$1.31 \pm 0.11$	$12.4 \pm 1.3$	$17.3 \pm 9.3$
	E2 (n=5)	$2.13 \pm 0.37^{**}$	$10.7 \pm 1.3$	$14.9 \pm 0.8$
7	Control (n=5)	$1.19 \pm 0.17$	$10.9 \pm 0.5$	$17.0 \pm 10.4$
	E2 (n=5)	$2.28 \pm 0.29^{**}$	$13.9 \pm 1.2$	$73.4 \pm 7.5$
	E2+HCN (n=5)	$1.84 \pm 0.27^*$	$15.2 \pm 1.0^*$	$62.9 \pm 12.0$
9	Control (n=5)	$1.18 \pm 0.15$	$10.4 \pm 0.3$	$32.3 \pm 17.8$
	E2 (n=5)	$1.89 \pm 0.11^*$	$18.2 \pm 1.8^{**}$	$107.6 \pm 14.1^{**}$
	E2 + HCN (n=5)	$1.56 \pm 0.33^*$	$18.8 \pm 2.2^{**}$	$154.7 \pm 54.3^{**}$
12	Control (n=5)	$1.49 \pm 0.27$	$10.5 \pm 0.8$	$42.5 \pm 11.0$
	E2 (n=5)	$2.13 \pm 0.18^{**}$	$14.8 \pm 3.6^*$	$147.4 \pm 97.5^*$
	E2+HCN (n=5)	$1.34 \pm 0.23$	$26.3 \pm 5.0^{**}$	$260.4 \pm 59.6^{**}$
17	Control (n=3)	$1.49 \pm 0.09$	$11.5 \pm 1.1$	$55.9 \pm 18.6$
	E2 (n=5)	$1.36 \pm 0.27$	$24.9 \pm 9.7^{**}$	$253.2 \pm 99.9^{**}$
	E2+HCN (n=5)	$1.23 \pm 0.22$	$21.3 \pm 2.6^{**}$	$144.6 \pm 20.2^{**}$

\*  $P > 0.05$ ; significantly different from controls.

\*\*  $P > 0.01$ ; significantly different from controls.

sample size = 4.

sample size = 3.

Table 9. Means and standard deviations in HSI, serum calcium, and serum phosphoprotein levels in rainbow trout following estradiol injections on days 0 and 3 of a 17-day experimental period in which exposure to 0.02 mg/L HCN started on day 4. Experiments were performed at 12.0 ± 1.0°C and the table contains the means of combined data of experiments performed in January and March, 1983. Note: E2 = estradiol-induced; E2 + HCN = estradiol-induced, cyanide-exposed.

Day sampled	Group	HSI (%)	Serum calcium (mg%)	Serum phosphoprotein phosphorus (ug/ml)
1	Control (n=10)	1.51 ± 0.21	8.5 ± 1.1	20.9 ± 25.5
	E2 (n=10)	1.62 ± 0.37	9.9 ± 0.9†	42.1 ± 17.2
4	Control (n=10)	1.40 ± 0.25	10.8 ± 2.2	45.4 ± 35.3
	E2 (n=10)	2.08 ± 0.26**	10.3 ± 1.0	71.5 ± 65.1
7	Control (n=10)	1.38 ± 0.28	11.5 ± 3.8	39.3 ± 26.7
	E2 (n=9)	2.53 ± 0.05**†	12.8 ± 1.4	63.1 ± 11.5**
	E2+HCN (n=10)	2.14 ± 0.37**	13.7 ± 2.0**	80.2 ± 36.7
9	Control (n=10)	1.17 ± 0.17	9.4 ± 1.3	47.7 ± 25.7
	E2 (n=9)	2.28 ± 0.31**	16.3 ± 2.0**	114.4 ± 27.8**
	E2 + HCN (n=10)	1.71 ± 0.38**	16.6 ± 2.5**	138.8 ± 29.4**†
12	Control (n=10)	1.34 ± 0.23	9.4 ± 1.3	47.7 ± 25.6
	E2 (n=9)	2.28 ± 0.31**	15.9 ± 4.2**	168.0 ± 98.4**
	E2+HCN (n=10)	1.71 ± 0.38	26.0 ± 4.4**	277.0 ± 69.8**
17	Control (n=9)	1.33 ± 0.24	10.9 ± 1.6	58.9 ± 24.2
	E2 (n=9)	1.42 ± 0.25	24.2 ± 7.4**	241.0 ± 107.9**
	E2+HCN (n=9)	1.38 ± 0.34	20.1 ± 3.2**	144.9 ± 26.0**

\* P > 0.05; significantly different from controls.

\*\* P > 0.01; significantly different from controls.

△ P > 0.05; significantly different from E2.

■ P > 0.01; significantly different from E2.

† sample size = 9.

than those of the estradiol-induced fish through to the twelfth day of the experiment, when mean HSI values were  $1.71 \pm 0.38 \%$  ( $n=10$ ) and  $2.28 \pm 0.31 \%$  ( $n=9$ ), respectively (Table 9). Both estradiol-induced cyanide-exposed and estradiol-induced groups declined to control values of  $1.38 \pm 0.34 \%$  ( $n=9$ ) and  $1.42 \pm 0.25 \%$  ( $n=9$ ) respectively by the seventeenth day of the experiment, and no significant ( $P < 0.05$ ) differences were observed between the two groups (Figure 11). Serum calcium and phosphoprotein phosphorus levels began to increase on the ninth day of the experiment. Mean serum levels in the estradiol-induced cyanide-exposed rainbow trout reached their peak levels on twelfth day of the experiment (Figure 11), with significantly ( $P > 0.01$ ) higher mean levels of  $26.0 \pm 4.4 \text{ mg\%}$  ( $n=8$ ) of calcium and  $277.0 \pm 69.8 \text{ }\mu\text{g/ml}$  ( $n=8$ ) of phosphoprotein phosphorus. Mean levels of serum calcium and phosphoprotein phosphorus in estradiol-induced rainbow trout were  $15.9 \pm 4.2 \text{ mg\%}$  ( $n=9$ ) and  $168.0 \pm 98.4 \text{ }\mu\text{g/ml}$  ( $n=8$ ) respectively. On day 17, however, mean serum calcium and phosphoprotein phosphorus levels had dropped to  $20.1 \pm 3.2 \text{ mg\%}$  ( $n=9$ ) and  $144.9 \pm 26.0 \text{ }\mu\text{g/ml}$  ( $n=9$ ) respectively in estradiol-induced cyanide-exposed fish, while levels of  $24.2 \pm 7.5 \text{ mg\%}$  ( $n=9$ ) and  $241.0 \pm 107.9 \text{ }\mu\text{g/ml}$  ( $n=9$ )



for serum calcium and phosphoprotein phosphorus were observed in estradiol-induced rainbow trout (Table 9).

The areas under the curve for estradiol-induced and estradiol-induced cyanide-exposed rainbow trout were calculated according to Simpson's Rule (Apple Statpak, 1982). The mean HSI of the estradiol-induced cyanide-exposed rainbow trout over the seventeen day experimental period was 12.2 % lower than that of the estradiol-induced fish, while mean serum calcium levels were 9.6 % higher and mean serum phosphoprotein phosphorus levels were 17.0 % higher in the estradiol-induced cyanide-exposed rainbow trout than in the estradiol-induced group.

Non-induced cyanide-exposed rainbow trout did not demonstrate any significant ( $P < 0.05$ ) differences in mean HSI, serum calcium, and serum phosphoprotein phosphorus levels from untreated control fish for the experiment conducted in January, 1983 (Figure 12). In the experiment conducted in March, 1983, however the mean HSI values for non-induced cyanide-exposed rainbow trout were significantly lower than those of untreated controls on days 12 and 17 of an experiment in which

Figure 12: Mean HSI, serum calcium, and serum phosphoprotein phosphorus levels in rainbow trout, Salmo gairdneri, ranging from 25 to 35 g, following exposure to 0.02 mg/L HCN for 13 days at  $12.0 \pm 1.0^{\circ}\text{C}$  during January, 1983.

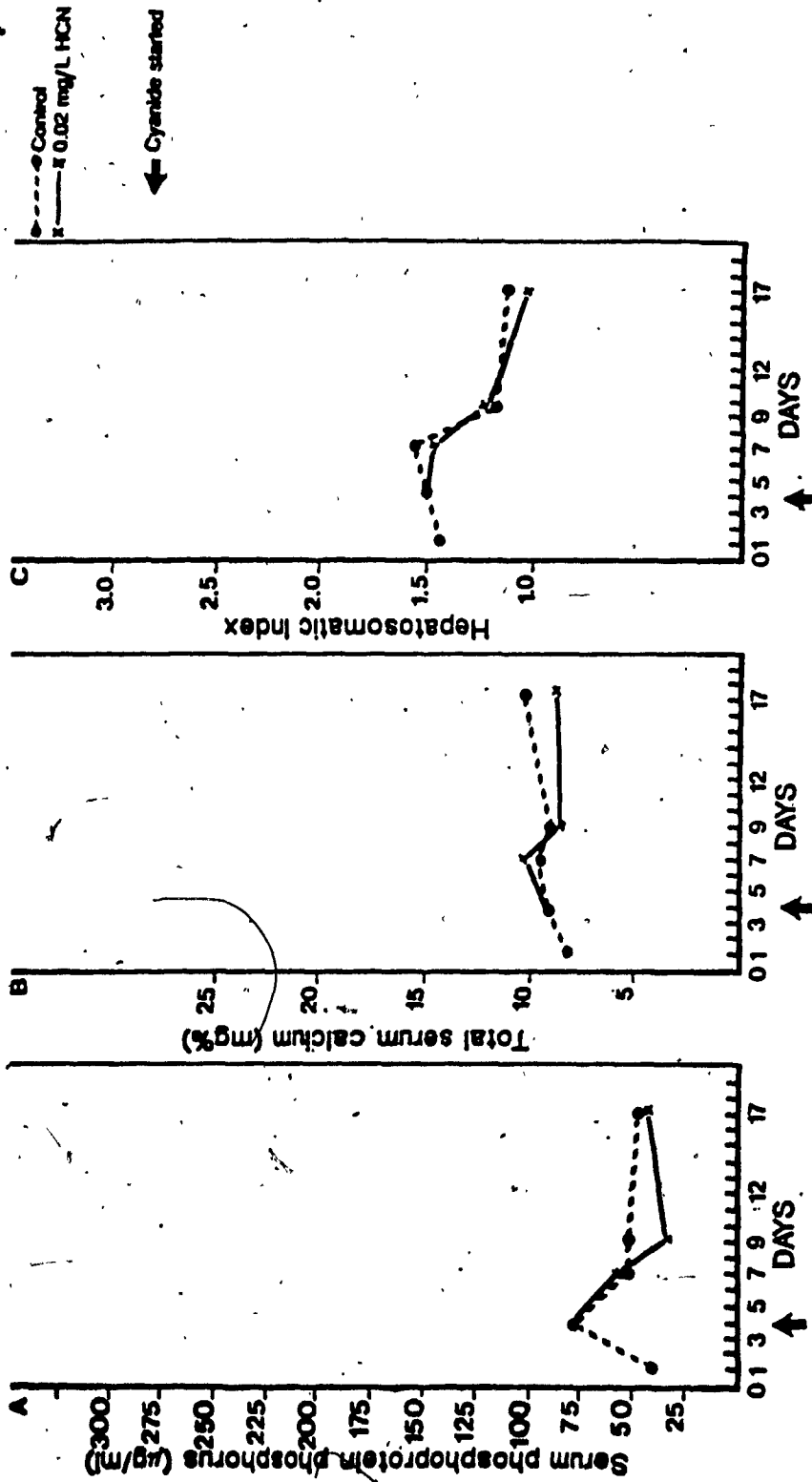


Figure 12

Table 10. Means and standard deviations of HSI, serum calcium, and serum phosphoprotein phosphorus levels in rainbow trout. Fish were exposed to 0.02 mg/L HCN from days 4 to 17 of a 17-day experiment. The experiment was performed at  $12.0 \pm 1.0^\circ\text{C}$  during January, 1983. No significant ( $P < 0.05$ ) between control and cyanide-exposed fish were observed.

Day Sampled	Group	HSI (%)	Serum Calcium (mg%)	Serum Phosphoprotein Phosphorus ( $\mu\text{g/ml}$ )
1	Control (n=5)	$1.40 \pm 0.24$	$8.1 \pm 0.5$	$40.0 \pm 27.7$
4	Control (n=5)	$1.49 \pm 0.26$	$9.1 \pm 0.8$	$76.4 \pm 16.4$
7	Control (n=5)	$1.57 \pm 0.11$	$9.6 \pm 0.8$	$57.2 \pm 14.9$
	Cyanide (n=5)	$1.43 \pm 0.18$	$10.1 \pm 0.4$	$53.1 \pm 16.3$
9	Control (n=5)	$1.16 \pm 0.11$	$8.8 \pm 0.7$	$52.0 \pm 6.9$
	Cyanide (n=5)	$1.20 \pm 0.02$	$8.7 \pm 0.3$	$37.3 \pm 4.$
17	Control (n=4)	$1.13 \pm 0.11$	$10.2 \pm 0.9$	$50.1 \pm 9.5$
	Cyanide (n=4)	$1.04 \pm 0.07$	$9.4 \pm 0.8$	$43.4 \pm 28.2$

Figure 13: Mean serum phosphoprotein phosphorus levels (a), serum calcium levels (b), and mean HSI (c) in rainbow trout, Salmo gairdneri, ranging from 25 to 35 g., following exposure to 0.02 mg/L HCN for 21 days at  $12.0 \pm 1.0$  °C, during March 1983.

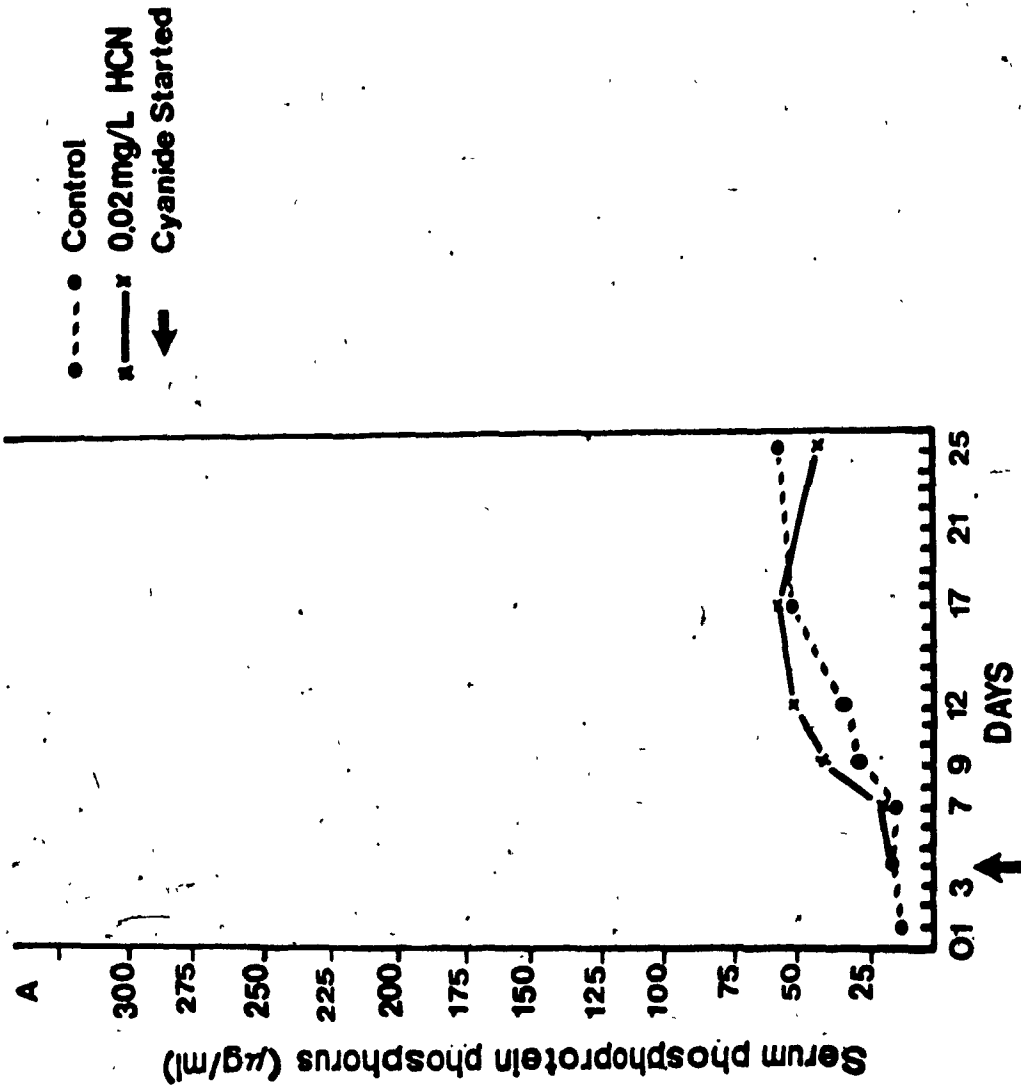


Figure 13a

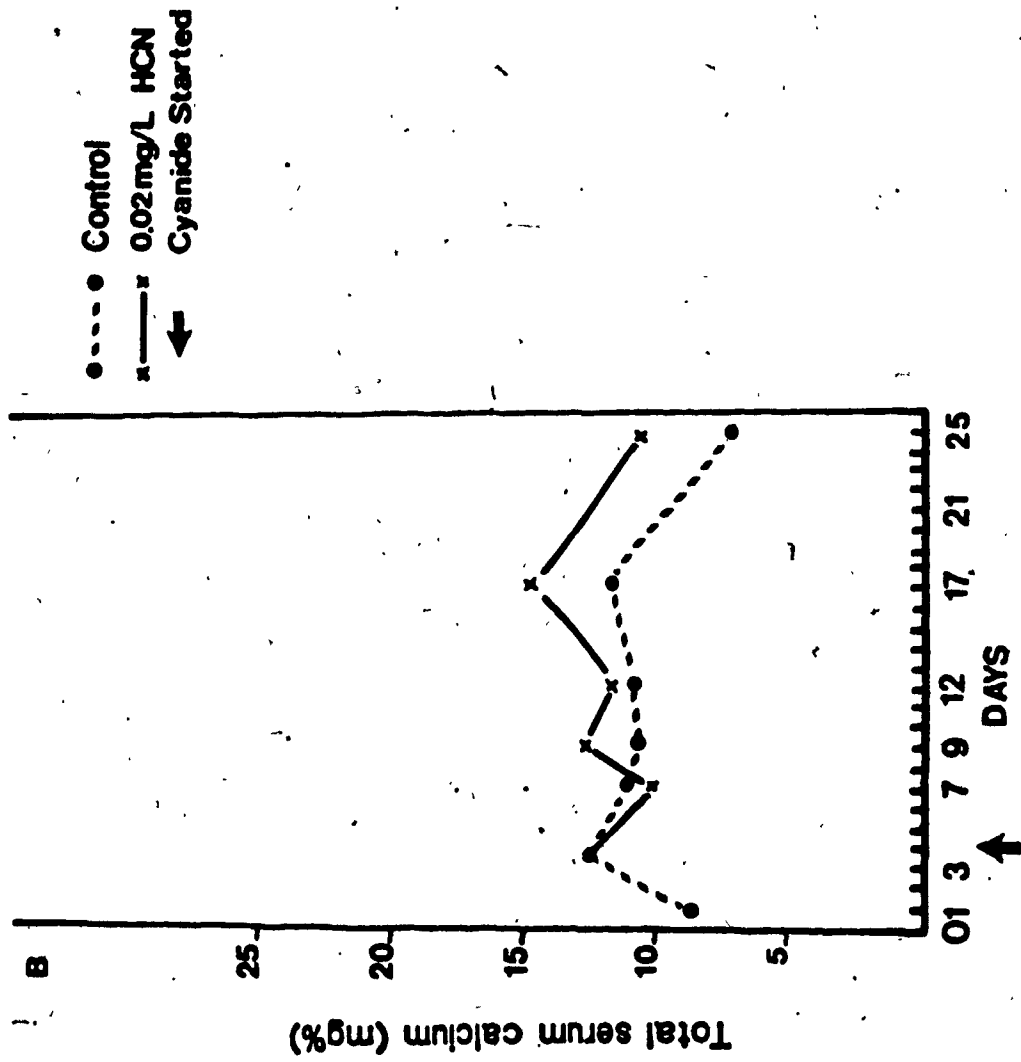


Figure 13 b

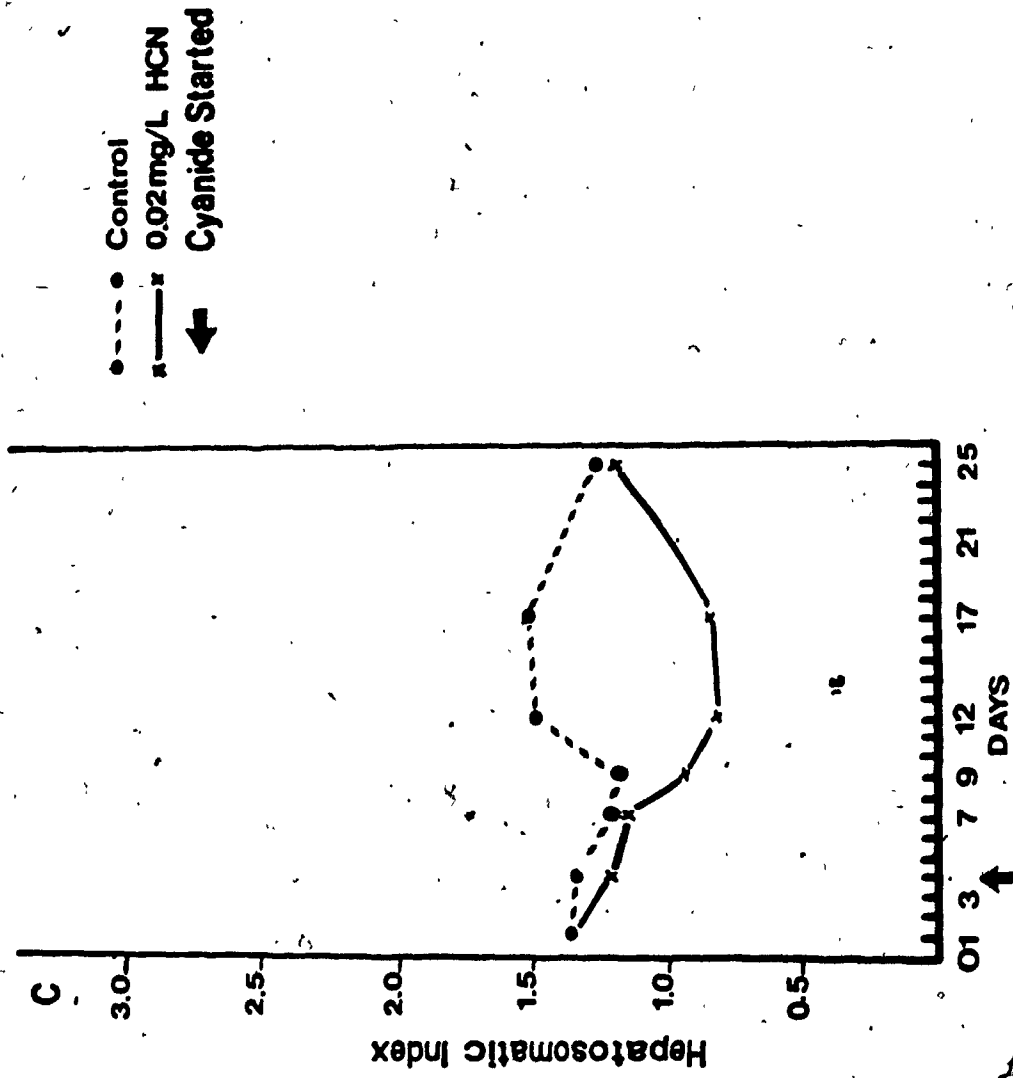


Figure 13 c



Table 11. Means and standard deviations of HSI, serum calcium, and serum phosphoprotein phosphorus levels in rainbow trout. Fish were exposed to 0.02 mg/L HCN from days 4 to 25 of a 25-day experiment performed at  $12.0 \pm 1.0^\circ\text{C}$  during March, 1983.

Day Sampled	Group	HSI (%)	Serum Calcium (mg%)	Serum Phosphoprotein Phosphorus (ug/ml)
1	Control (n=5)	1.43 $\pm$ 0.10	8.9 $\pm$ 0.8	14.0 $\pm$ 2.0
4	Control (n=5)	1.31 $\pm$ 0.11	12.4 $\pm$ 1.3	17.3 $\pm$ 9.3
7	Control (n=5)	1.19 $\pm$ 0.17	10.9 $\pm$ 0.5	17.0 $\pm$ 10.4
	Cyanide (n=5)	1.16 $\pm$ 0.12	10.0 $\pm$ 1.2	24.0 $\pm$ 12.0
9	Control (n=5)	1.18 $\pm$ 0.15	10.4 $\pm$ 0.3	32.3 $\pm$ 17.8
	Cyanide (n=5)	0.92 $\pm$ 0.09*	12.3 $\pm$ 1.1	47.1 $\pm$ 10.7
12	Control (n=5)	1.49 $\pm$ 0.27	10.5 $\pm$ 0.8	42.5 $\pm$ 11.0
	Cyanide (n=5)	0.84 $\pm$ 0.16*	11.3 $\pm$ 0.3	51.5 $\pm$ 4.9
17	Control (n=5)	1.49 $\pm$ 0.09	11.5 $\pm$ 1.1	55.9 $\pm$ 18.6
	Cyanide (n=5)	0.87 $\pm$ 0.12*	14.4 $\pm$ 2.0	62.4 $\pm$ 14.8
25	Control (n=3)	1.23 $\pm$ 0.07	7.3 $\pm$ 0.09	62.9 $\pm$ 1.1
	Cyanide (n=5)	1.17 $\pm$ 0.06	10.4 $\pm$ 2.3	38.8 $\pm$ 12.3

\* P > 0.05; significantly different from controls.

cyanide exposure started on day 4 and ended on day 25 (Figure 13). Mean HSI values of  $0.84 \pm 0.16 \%$  ( $n=5$ ) and  $0.87 \pm 0.12 \%$  ( $n=5$ ) were observed in cyanide-exposed rainbow trout on days 12 and 17 respectively, while mean levels of  $1.47 \pm 0.27 \%$  ( $n=5$ ) and  $1.49 \pm 0.09 \%$  ( $n=5$ ) respectively, were observed in untreated control fish (Table 11). The mean HSI values in non-induced cyanide-exposed rainbow trout increased to  $1.19 \pm 0.06 \%$  ( $n=5$ ); on day 22 the control mean was  $1.23 \pm 0.09 \%$  ( $n=3$ ) (Table 11). No significant ( $P < 0.05$ ) differences were observed in mean serum calcium and serum phosphoprotein phosphorus levels between non-induced cyanide-exposed and untreated control rainbow trout (Figure 13).

The data was then examined according to the sex of the fish being sampled in order to determine any differences in the response of male and female rainbow trout. Estradiol-induced cyanide-exposed male and female rainbow trout did not demonstrate any significant ( $P < 0.05$ ) differences in serum calcium and phosphoprotein phosphorus levels (Figure 14). Serum calcium and phosphoprotein phosphorus levels in male and female rainbow trout reached their peak levels by day 12, with

Figure 14: Changes in mean HSI, serum calcium, and serum phosphoprotein phosphorus levels in separate male and female rainbow trout, Salmo gairdneri, which received estradiol injections on days 0 and 3 of a 17 day experimental period. Fish were sampled on days 1, 4, 7, 9, 12, and 17. Experiments were performed at  $12.0 \pm 1.0^{\circ}\text{C}$  and the graphs represent combined data from two experiments performed in January and March, 1983.

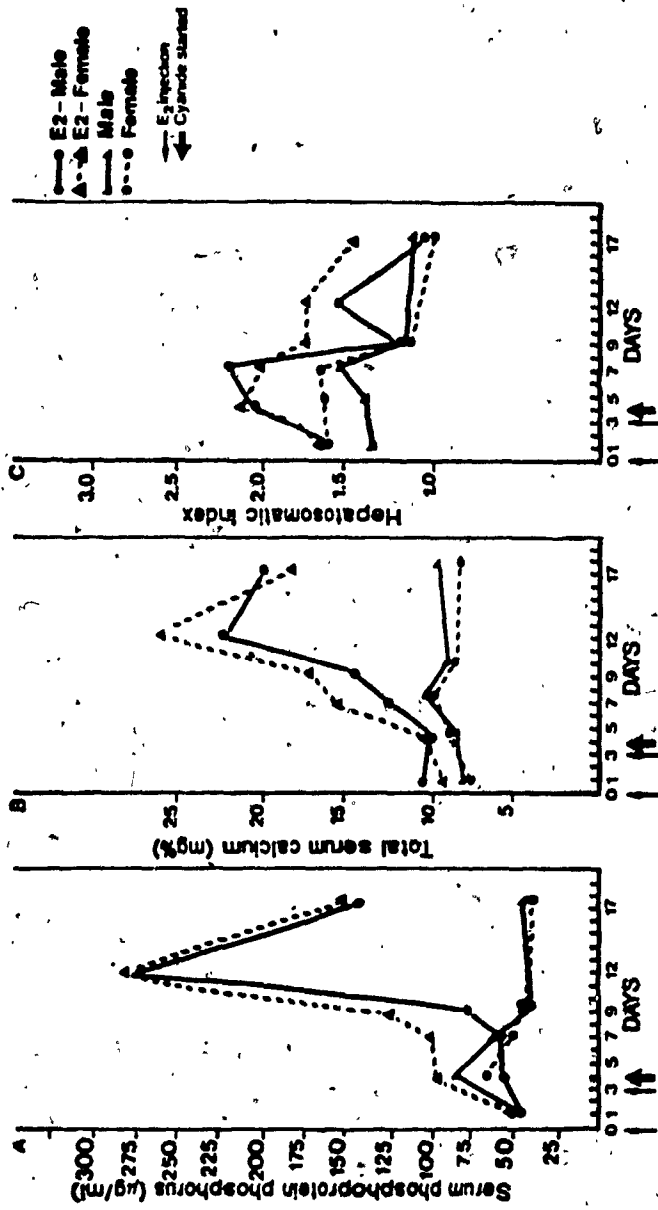


Figure 14

Table 12. Means and standard deviations of HSI, serum calcium, and serum phosphoprotein phosphorus levels in separate male and female rainbow trout which received estradiol injections on days 0 and 3 of a 17-day experimental period in which exposure to 0.02 mg/L HCN started on day 4. Fish were sampled on days 1, 7, 9, 12, and 17. Experiments were performed at  $12.0 \pm 1.0^\circ\text{C}$  and the table contains the combined data from two experiments conducted in January and March, 1983. No significant ( $P < 0.05$ ) differences were observed between male and female fish.

Day Sampled	Group	HSI (%)	Serum Calcium (mg%)	Serum Phosphoprotein Phosphorus (ug/ml)
1	Male (n=6)	1.41 $\pm$ 0.38	10.6 $\pm$ 0.8	39.9 $\pm$ 17.2
	Female (n=3)	2.41 $\pm$ 0.50	9.0 $\pm$ 0.9	53.7 $\pm$ 43.3
4	Male (n=7)	2.06 $\pm$ 0.32	10.2 $\pm$ 1.0	41.3 $\pm$ 42.5
	Female (n=3)	2.15 $\pm$ 0.28	10.3 $\pm$ 0.8	95.4 $\pm$ 55.4
7	Male (n=4)	2.21 $\pm$ 0.29	12.0 $\pm$ 2.0	44.3 $\pm$ 17.6
	Female (n=4)	2.03 $\pm$ 0.45	15.3 $\pm$ 1.0	104.0 $\pm$ 83.7
9	Male (n=2)	1.27 $\pm$ 0.19	14.3 $\pm$ 3.8	78.2
	Female (n=7)	1.84 $\pm$ 0.19	17.2 $\pm$ 1.3	127.6 $\pm$ 3.6
12	Male (n=5)	1.55 $\pm$ 0.35	22.8 $\pm$ 5.5	275.3 $\pm$ 67.3
	Female (n=5)	1.88 $\pm$ 0.37	25.9 $\pm$ 3.4	278.1 $\pm$ 53.4
17	Male (n=2)	1.09 $\pm$ 0.23	19.9 $\pm$ 3.5	132.1 $\pm$ 3.8
	Female (n=7)	1.46 $\pm$ 0.21	18.0 $\pm$ 4.4	148.4 $\pm$ 36.0

mean values of  $22.8 \pm 5.5$  mg% (n=6) and  $275.3 \pm 67.3$   $\mu\text{g/ml}$  (n=3) respectively for the male fish and  $25.9 \pm 3.4$  mg% (n=3) and  $278.1 \pm 53.4$   $\mu\text{g/ml}$  (n=5) respectively for the female fish (Table 12). Mean HSI values in estradiol-induced cyanide-exposed male rainbow trout were lower than in the females. Mean HSI values of  $1.27 \pm 0.19$  % (n=2),  $1.55 \pm 0.35$  % (n=7), and  $1.09 \pm 0.23$  % (n=2) were observed in male rainbow trout on days 9, 12, and 17 of the seventeen day experiments. Mean HSI values in female rainbow trout over the same experimental period were  $1.84 \pm 0.19$  % (n=7),  $1.88 \pm 0.37$  % (n=5), and  $1.46 \pm 0.21$  % (n=7) respectively (Table 12). Estradiol-induced rainbow trout did not demonstrate any significant ( $P < 0.05$ ) differences between males and females for HSI, serum calcium, and serum phosphoprotein phosphorus levels (Figure 15). Serum calcium and phosphoprotein phosphorus levels in estradiol-induced males peaked on day 17, with a mean serum calcium level of  $23.0 \pm 5.4$  mg% (n=6) while the mean calcium levels for female rainbow trout was  $26.5 \pm 10.5$  mg% (n=3) (Table 13). Mean serum phosphoprotein phosphorus levels on day 17 for male and female rainbow trout were  $239.6 \pm 91.2$   $\mu\text{g/ml}$  (n=6) and  $243.9 \pm 115.7$   $\mu\text{g/ml}$  (n=3), respectively (Table 13). Mean HSI values peaked on day 7, with values

Figure 15: Changes in mean HSI, serum calcium, and serum phosphoprotein phosphorus levels in estradiol-induced male and female rainbow trout, Salmo gairdneri, when separated, exposed to a sublethal concentration of 0.02 mg/L of cyanide starting on day 4. Fish were sampled on days 1, 4, 7, 9, 12, and 17. Experiments were performed at 12.0 ± 1.0 C and the graphs represent combined data from two experiments performed in January and March, 1983.

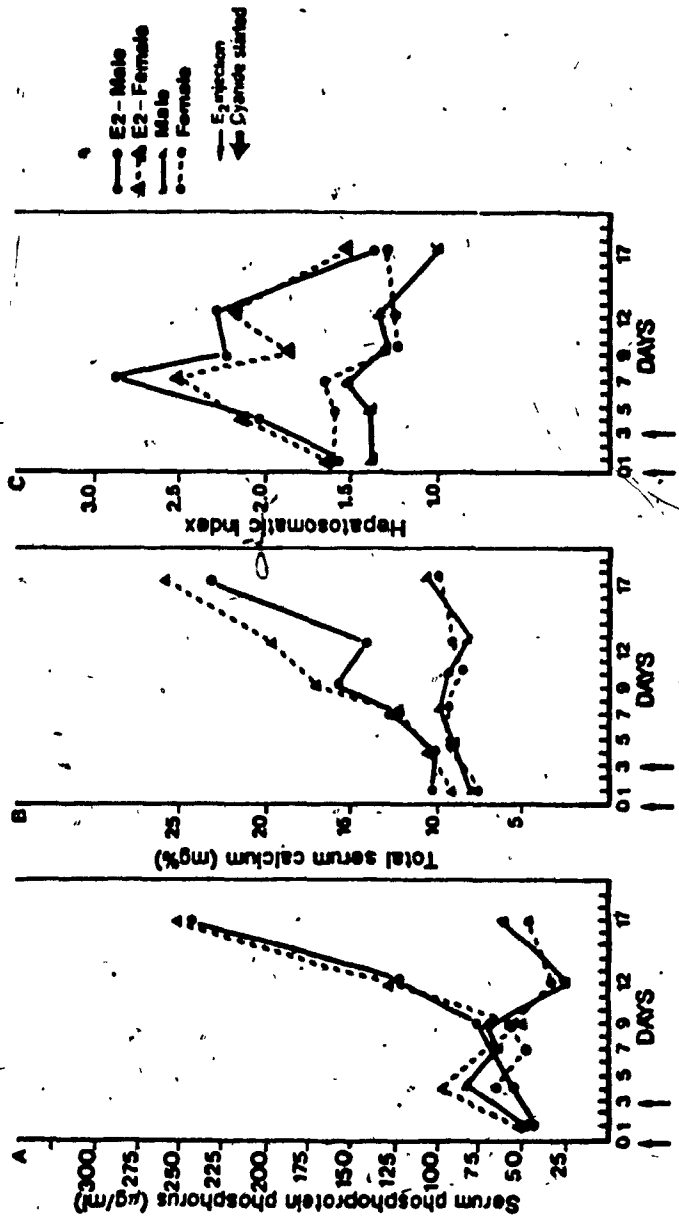


Figure 15



Table 13. Means and standard deviations of HSI, serum calcium, and serum phosphoprotein phosphorus levels in separate male and female rainbow trout which were injected with 5 mg/kg of body weight of estradiol on days 0 and 3 of a 17-day experimental period. Fish were sampled on days 1, 4, 7, 9, 12, and 17. Experiments were performed at  $12.0 \pm 1.0^\circ\text{C}$  and the table contains combined data from two experiments conducted in January and March, 1983. No significant ( $P < 0.05$ ) differences were observed between male and female fish.

Day Sampled	Group	HSI (%)	Serum Calcium (mg%)	Serum Phosphoprotein Phosphorus (ug/ml)
1	Male (n=6)	$1.61 \pm 0.38$	$10.6 \pm 0.8$	$39.9 \pm 17.2$
	Female (n=3)	$2.41 \pm 0.50$	$9.0 \pm 0.9$	$53.7 \pm 43.3$
4	Male (n=7)	$2.06 \pm 0.32$	$10.2 \pm 1.0$	$61.3 \pm 62.5$
	Female (n=3)	$2.15 \pm 0.28$	$10.3 \pm 0.8$	$95.4 \pm 55.4$
7	Male (n=1)	2.85	12.9	77.3
	Female (n=8)	$2.48 \pm 0.48$	$12.8 \pm 1.1$	$61.3 \pm 8.2$
9	Male (n=7)	$2.28 \pm 0.25$	$15.9 \pm 1.9$	$124.9 \pm 30.8$
	Female (n=3)	$1.82 \pm 0.14$	$17.4 \pm 2.3$	$130.6 \pm 30.1$
12	Male (n=6)	$2.31 \pm 0.33$	$14.0 \pm 2.8$	$136.2 \pm 69.5$
	Female (n=3)	$2.20 \pm 0.41$	$19.6 \pm 3.5$	$221.2 \pm 72.2$
17	Male (n=4)	$1.34 \pm 0.19$	$23.0 \pm 5.4$	$239.6 \pm 91.2$
	Female (n=3)	$1.52 \pm 0.41$	$26.5 \pm 10.5$	$243.9 \pm 115.7$

of 2.85 (n=1) for males and  $2.48 \pm 0.48$  (n=8) for females (Table 13).

To determine the effects of sublethal cyanide (0.02 mg/L) pre-exposure on exogenous yolk production, immature rainbow trout were pre-exposed to cyanide for thirteen days prior to receiving the first of two estradiol injections on days 14 and 17. The fish were sampled on day 21. In this experiment, no significant ( $P < 0.05$ ) differences were observed in mean HSI values between estradiol-induced and estradiol-induced cyanide exposed rainbow trout (Figure 16). The mean HSI values on day 7 of the experiment were observed to be  $1.74 \pm 0.26$  % (n=4) and  $2.41 \pm 0.35$  % (n=3), for estradiol-induced cyanide exposed and estradiol-induced rainbow trout, respectively. Both groups demonstrated significantly ( $P > 0.05$ ) higher mean values than untreated control rainbow trout which displayed a mean HSI of  $1.23 \pm 0.06$  % (n=3) (Table 14). Serum calcium levels were not significantly ( $P < 0.05$ ) different between estradiol-induced and estradiol-induced cyanide-exposed rainbow trout (Figure 16), which exhibited mean levels of  $13.8 \pm 3.4$  mg% (n=2) and  $13.1 \pm 0.44$  mg% (n=3) respectively. Once again, both groups were significantly

Figure 16: Histogram of mean values for HSI, serum calcium, and serum phosphoprotein phosphorus levels in rainbow trout, Salmo gairdneri, pre-exposed for 13 days to a sublethal concentration of 0.02 mg/L HCN prior to estradiol injections on days 14 and 17 of a 21 day experimental period. Fish were sampled on day 21 and the experiment was performed at  $12.0 \pm 1.0^{\circ}\text{C}$ . The experiment was conducted in March, 1983.

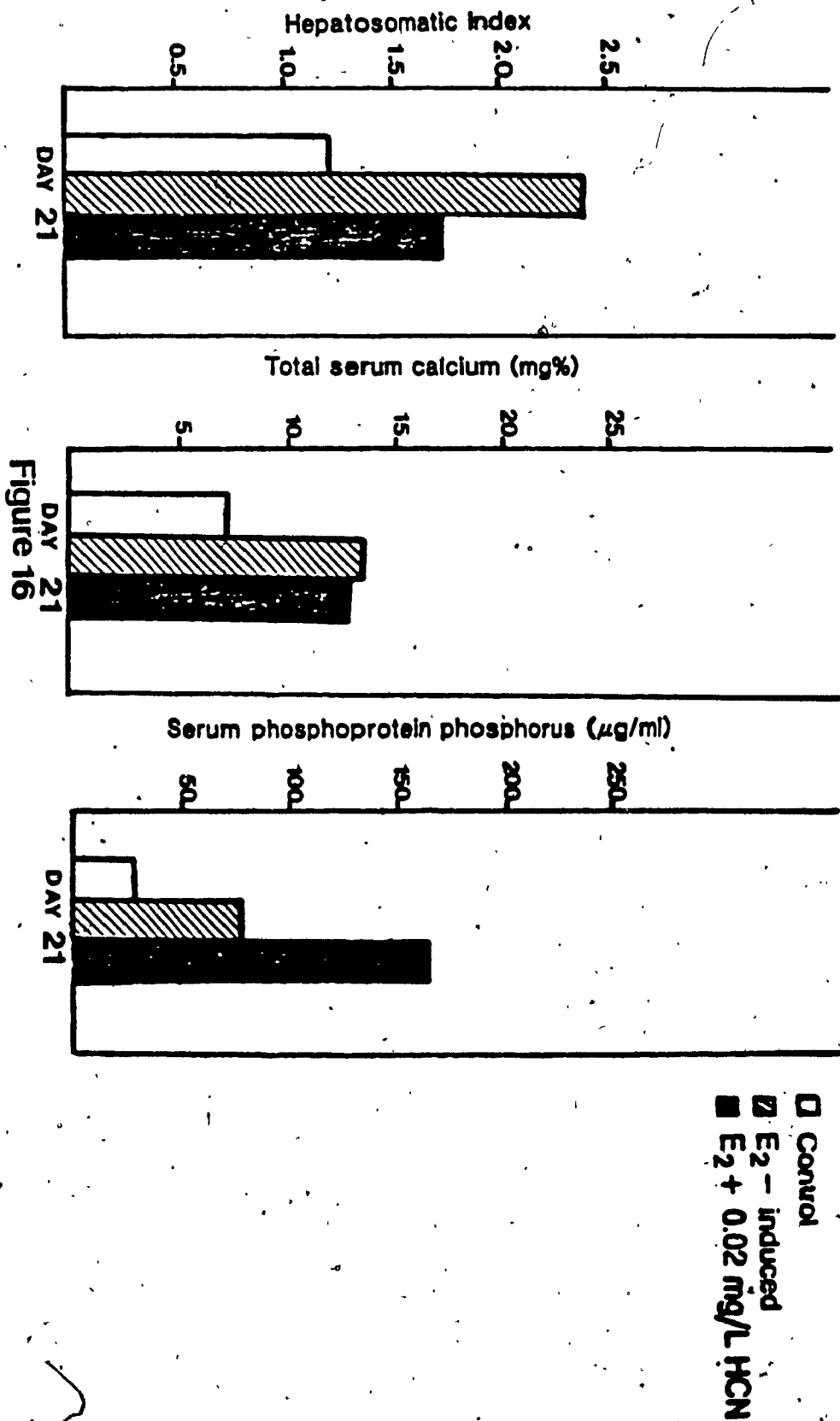


Table 14. Means and standard deviations of HSI, serum calcium, and serum phosphoprotein phosphorus levels in rainbow trout pre-exposed to 0.02 mg/L HCN for 13 days prior to estradiol (5 mg/kg) injections on days 14 and 17 of a 21 day experiment. Fish were sampled on day 21 and the experiment was performed at  $12.0 \pm 1.0^\circ\text{C}$  during March, 1983.

Day Sampled	Group	HSI (%)	Serum Calcium (mg%)	Serum Phosphoprotein Phosphorus ( $\mu\text{g/ml}$ )
21	Control (n=3)	$1.22 \pm 0.07$	$7.2 \pm 0.9$	$47.8 \pm 20.1$
	E2 (n=3)	$2.41 \pm 0.50^*$	$13.8 \pm 3.4^*$	77.9
	E2 + HCN(n=4)	$1.74 \pm 0.26^*$	$13.1 \pm 0.4^*$	$167.2 \pm 4.5^{**}$

\*  $P > 0.05$ ; significantly different from controls.

No significant ( $P < 0.05$ ) differences were observed between estradiol-induced and estradiol-induced cyanide-exposed fish.

( $P > 0.05$ ) higher than untreated control rainbow trout which were observed to have a mean calcium level of  $7.2 \pm 0.9$  mg% (n=3) (Table 14). Serum phosphoprotein phosphorus levels in estradiol-induced cyanide-exposed rainbow trout were not significantly ( $P < 0.05$ ) different from estradiol-induced fish with mean levels of  $77.9 \mu\text{g/ml}$  (n=1) and  $167.2 \pm 6.5 \mu\text{g/ml}$  (n=3), respectively, due to the small sample size of the estradiol-induced rainbow trout (Table 14). Estradiol-induced cyanide exposed rainbow trout demonstrated significantly ( $P > 0.05$ ) higher mean levels than untreated control rainbow trout, the latter having a mean phosphoprotein phosphorus level of  $47.8 \pm 20.1 \mu\text{g/ml}$  (n=3) (Table 14):

## Discussion

Immature rainbow trout, Salmo gairdneri, injected with B-estradiol-3-benzoate and exposed to a sublethal concentration of 0.02 mg/L of cyanide on days 0 or 4, demonstrated higher levels of exogenous yolk on the twelfth day of a 17-day experimental period. While levels of exogenous yolk were higher on day-12 when the fish were exposed to cyanide, the amount of exogenous yolk present in the serum over the 17-day experimental period did not appear to be altered by cyanide. It was also observed in this study that a seasonal difference exists with respect to the effects of sublethal cyanide exposure on exogenous yolk production in rainbow trout. This was demonstrated by an experiment performed in June, in which sublethal exposure to cyanide did not cause any significant effects on exogenous yolk production. However, when the experiment was repeated in October, cyanide exposure was found to cause higher levels of exogenous yolk on day-12. It was therefore concluded that exposure to 0.02 mg/L. of cyanide alters the patterns of exogenous yolk production and release in rainbow trout.

In this study it was demonstrated that serum calcium levels were not altered when blood samples were taken either by heart puncture or from the dorsal aorta by caudal severance (Table 1). Serum phosphoprotein phosphorus, however, did demonstrate higher levels in blood samples taken by caudal severance although these differences were not significant. These results support previous findings by Thurston (1967), who reported elevated total serum protein levels in rainbow trout which had been bled by caudal severance as compared to heart puncture: While Thurston studied total protein levels, phosphoprotein phosphorus levels which make up only a portion of the total proteins, were used in this study. The results of this study therefore indicate that the phosphoprotein fraction of the total serum proteins is being altered in blood removed by caudal severance. A possible explanation for this is that some phosphoproteins may be liberated from the severed muscle as a result of the physical damage to the muscles. The fish used in this study were bled by caudal severance. The caudal severance technique was chosen in preference to heart puncture because of the



high level of hemolysis (40 %) and smaller volumes of blood obtained by the latter procedure. Levels for heart puncture ranged from 50 to 100  $\mu$ l, as compared to blood volumes of 250 to 400  $\mu$ l obtained by caudal severance. For the purpose of this study, it was assumed that any phosphoprotein phosphorus contamination was equivalent amongst the various fish groups being sampled.

Based on previous reports in the literature, peanut oil was selected as the carrier for estradiol injections because of its lipophilic physical properties. This permitted estradiol to go into solution, and enabled it to be rapidly absorbed by the fish. Injections of peanut oil did not cause any significant differences in HSI, serum calcium, and serum phosphoprotein phosphorus levels in this study. Terkatin-Shimony and Yaron (1978) demonstrated that estradiol was rapidly absorbed in 1.5 kg ovariectomized female Tilapia aurea, following a 1.3 ml/kg intraperitoneal injection of 0.5 mg estradiol<sup>17 $\beta$</sup>  dissolved in sesame oil (a carrier similar to peanut oil). Their experiments demonstrated that estradiol was absorbed by the fish

within 5 hours following a single injection, and that serum estradiol levels of 1 ng/ml were still detectable 315 hours following the injection. Furthermore, Campbell and Idler (1980) demonstrated that immature rainbow trout (20-25 g) could be induced to produce vitellogenin using repetitive injections of 5 mg/kg/0.5 ml of estradiol dissolved in peanut oil.

An estradiol dosage of 5 mg/kg of body weight was selected for these experiments according to levels previously reported in the literature. Using this concentration, Elliot et al. (1979) demonstrated that juvenile (200-g) rainbow trout could be induced to produce exogenous yolk following a single estradiol injection, with serum calcium and serum phosphoprotein phosphorus levels being used as indicators of exogenous yolk. Similarly, Campbell and Idler (1980) induced yolk production, as measured by vitellogenin production, in immature (20-25 g) rainbow trout using four 50  $\mu$ l injections of 5 mg/kg of estradiol benzoate administered every three days during a twelve-day experimental period.

As in the work of Elliot et al. (1979), our results indicate that exogenous yolk production was induced in estradiol-injected immature rainbow trout. This was based on estradiol-induced rainbow trout which displayed significantly ( $P > 0.01$ ) higher serum calcium and serum phosphoprotein phosphorus levels from days 9 to 17 of a seventeen-day experimental period (Figure 7).

The hepatosomatic index was selected as a broad indicator of change in liver activity since this is the site of exogenous yolk synthesis. In naturally reproducing sexually mature female rainbow trout, van Bohemen et al. (1981) observed that the hepatosomatic index increased from March to December, with respective values of  $1.2 \pm 0.1 \%$  to  $3.0 \pm 0.3 \%$ . Estradiol-induced immature rainbow trout in this study demonstrated elevated HSI values which peaked on day 7, with values of  $2.53 \pm 0.50 \%$ , following estradiol injections on days 0 and 4, in contrast to a mean control value of  $1.38 \pm 0.28 \%$  (Figure 7). The elevated HSI values can be attributed to increased levels of RNA formation in the liver as a result of estradiol induction. Korsgaard et al. (1983) have

shown that male flounder, Platichthys flesus (L), demonstrate increases in cellular content of bulk RNA in the liver following the administration of estradiol, while total liver proteins remain unchanged. The mean HSI values subsequently declined on day 9 and increased to a second peak on day 12 (Figure 7). This decline and subsequent increase may reflect a decline and increase in serum estradiol levels caused by the time lapse between the first and the second estradiol injection. Korsgaard et al. (1983) have shown that continuous action of estradiol on the liver is necessary to maintain maximum activity of the translational apparatus involved in the production of bulk liver RNA.

Serum calcium and serum phosphoprotein phosphorus levels began to increase on day 7 and continued to increase throughout the 17-day experiment (Table 3). The serum levels in this study were within the physiological range reported by Whitehead and Bromage (1978) for sexually mature vitellogenic female rainbow trout. In their study on the annual production of exogenous yolk in vitellogenic female trout, Whitehead and Bromage (1978) observed that

serum calcium and serum phosphoprotein phosphorus levels steadily increased from April to February, with serum levels ranging from  $12.0 \pm 0.3$  mg% and  $15.6 \pm 8.6$  ug/ml in April to  $69.2 \pm 9.7$  mg% and  $481.3 \pm 61.1$  ug/ml in December respectively. Serum calcium and serum phosphoprotein phosphorus levels in this study began to increase only after day 7 following mean peak HSI values. This suggests that the peak level of HSI represented exogenous yolk precursors that were subsequently released into the serum and detected during sampling on day 9, thereby accounting for both the increase in serum calcium and phosphoprotein phosphorus and the decrease in HSI. This also suggests that the liver builds up its yolk precursors prior to their release into the serum. It is therefore possible that there are two physiological factors which act upon the liver during exogenous yolk production. One of these factors, estradiol, may be responsible for inducing the translational apparatus involved in RNA synthesis while a second factor, which has not yet been identified, may be involved in stimulating the release of exogenous yolk from the liver. There is some evidence in the literature that this second

factor may be estrone. van Bohmen and Lambert (1981) have shown that in sexually mature female rainbow trout undergoing vitellogenesis, the annual cycle of estradiol peaks one month prior to maximum vitellogenin levels which peak simultaneously with estrone levels. This suggests that in sexually mature female rainbow trout undergoing vitellogenesis, estradiol may be stimulating the production of exogenous yolk precursors in the liver one month prior to their assembly and release into the serum. The concerted increases in serum vitellogenin and estrone levels also suggest that estrone may be stimulating the release of vitellogenin into the serum. Further studies by van Bohmen et al. (1982) in which immature (200-250 g) rainbow trout were injected with estradiol and estrone mixtures of varying concentrations and ratios once a day for 7 days, demonstrated that plasma vitellogenin levels were higher in fish which had been injected with an estradiol to estrone ratio of 1:3 at a dosage of 250 ng/g, as compared to rainbow trout which had been administered only estradiol at dosages of 100, 250, and 500 ng/g. This implies that the estrone within the mixture could be acting upon the liver to

stimulate a faster release of vitellogenin from the liver. While estradiol alone was injected into the immature rainbow trout in this study, it is possible that the estradiol is metabolized to estrone, which might then initiate the release of exogenous yolk. Hanson and Rafter (1983) have shown that estradiol-17 $\beta$  can be metabolized to estrone and two estriols (estra-1,3,5(10)-triene,16 $\beta$ ,17 $\beta$ ,triol and estra-1,3,5(10)-triene,3,7 $\alpha$ ,17 $\beta$ -triol) in vitro by the liver microsomes of rainbow trout. This conversion of estradiol-17 $\beta$  to estrone and estriol is catalyzed by the enzyme 17-hydroxysteroid oxidoreductase, which hydroxylates the estradiol-17 $\beta$  to form estrone and estriol. The dual intracellular actions of estradiol and estrone upon the hepatic estrogen receptor have been demonstrated by Turner et al. (1981). They showed that estrone replaces estradiol from its intracellular hepatic receptor in vitro in Pacific hagfish, Eptatretus stouti, as a result of the higher affinity of estrone for the estrogen receptor. It is therefore possible that following induction of protein synthesis, as a result of the estradiol-receptor complex binding to the chromatin of the DNA (Grody et al., 1982), the

estradiol is displaced from its receptor by estrone, which subsequently initiates the release of exogenous yolk from the liver. This may explain why exogenous yolk is being released only after day 7, which would represent the time required to metabolize the estradiol and initiate the release of exogenous yolk from the liver.

Campbell and Idler (1980) have shown that exogenous yolk, or vitellogenin, in rainbow trout is a glycolipophosphoprotein-calcium complex with a molecular weight of 455,000 daltons, containing 0.6 % phosphorus and one calcium atom per vitellogenin complex. It is therefore possible to measure exogenous yolk indirectly using serum calcium and phosphoprotein phosphorus levels. The strong correlation ( $r=0.98$ ) between mean serum calcium and phosphoprotein phosphorus levels observed in this study for estradiol-induced immature rainbow trout suggests that the increases in these serum parameters are indicative of the presence of exogenous yolk. Further analysis of the serum samples by Dr. D.R. Idler (Director of Marine Sciences Research Laboratory, Memorial University of Newfoundland) has



confirmed the presence of vitellogenin in serum obtained from estradiol-induced immature rainbow trout (Appendix 1). A strong correlation ( $r=0.95$ ) between serum calcium and serum phosphoprotein phosphorus levels has also been observed by Whitehead et al. (1978) in their study dealing with the annual production of exogenous yolk in naturally reproducing vitellogenic female rainbow trout.

In the first series of experiments, a seasonal difference was observed with respect to HSI, serum calcium, and serum phosphoprotein phosphorus levels in estradiol-induced cyanide-exposed rainbow trout for experiments performed in June and October, 1983. In these experiments estradiol-induced immature rainbow trout were exposed to a sublethal cyanide concentration of 0.02 mg/L beginning on day 0 of a 17 day experimental period. This provided an opportunity to study the effects of cyanide on the production of exogenous yolk in the liver, since cyanide exposure began simultaneously with the estradiol injections. For the experiment performed in June, no significant differences were observed in HSI, serum calcium, and serum phosphoprotein phosphorus levels between

estradiol-induced and estradiol-induced cyanide-exposed rainbow trout (Figure 8). When the experiment was repeated in October, the fish displayed a response similar to that observed in rainbow trout exposed to 0.02 mg/L of cyanide starting on day 4. The HSI of the estradiol-induced cyanide-exposed rainbow trout were lower than those of the estradiol-induced fish. Serum calcium and phosphoprotein phosphorus levels in estradiol-induced cyanide-exposed rainbow trout both peaked on day 12 with significantly higher mean levels than in estradiol-induced rainbow trout (Table 6). Mean serum levels subsequently decreased by day 17, for the estradiol-induced cyanide-exposed group, while mean levels in the estradiol-induced group were still increasing by day 17 (Figure 10). The different responses, in the three parameters studied, obtained during these two experiments suggest a possible seasonal difference in response to sublethal cyanide toxicity. Other investigators have reported seasonal differences in the acute toxicity of cyanide. Our results concur with those reported by McGeachy and Leduc (1983) who demonstrated, using fingerling rainbow trout, that the 96 hr LC50 for cyanide was

higher in the summer than in the winter months. This suggests that the sensitivity of rainbow trout to cyanide is decreased during the summer, thereby accounting for the observations of the present study for the experiment conducted in the month of June. A possible explanation for these seasonal differences is that these fish underwent changes in natural hormonal cycles. While this study has been performed using immature rainbow trout in order to overcome reproductive cycles, which have been observed in sexually mature fish (Bromage et al., 1982), it is possible that immature fish undergo non-reproductive hormonal cycles such as those of thyroid hormones. Singh (1977) reported that thyroid activity in the catfish, Mystus vittatus (Bloch), as measured by  $I^{131}$  uptake, begins to increase during the months of February and March and decreases during September. It is possible that such a cycle could influence the response of the fish and thereby account for the seasonal differences.

A second possible explanation is that the fish used in these two experiments were hatched at the same time and that in order to obtain the same size of

fish the June fish could be characterized as "fast growing" fish while those used in October would be the "slow growing" fish. As a result of these fish demonstrating different growth rates, their metabolic rates would also be different; i.e. faster in the "fast growing" fish and slower in the "slow growing" fish. It is therefore possible that these different metabolic rates could account for differences in sensitivity to sublethal cyanide exposure. The seasonal difference observed in estradiol-induced cyanide-exposed fish is an important factor to consider when using this experimental model. While these seasonal differences may be inherent to cyanide and its mechanism of toxicity, other toxicants may also display similar seasonal differences and therefore could lead to erroneous conclusions, if experiments are conducted at a time when the fish are less sensitive to the toxicant. Non-induced cyanide-exposed rainbow trout did not demonstrate any significant differences, from controls, in HSI, serum calcium, and serum phosphoprotein phosphorus levels for the same experiment. This suggests that the effects of cyanide on serum calcium, serum phosphoprotein phosphorus, and HSI observed in

estradiol-induced cyanide-exposed rainbow trout are in fact specific effects upon exogenous yolk, and not general effects on these parameters.

A second series of experiments, performed during the months of January and March, dealt with the effects of cyanide on the final stages of yolk synthesis in the liver and its release into the serum. Estradiol-induced immature rainbow trout were exposed to a sublethal cyanide concentration of 0.02 mg/L starting on day 4 of a seventeen day experimental period. The experimental design thus evaluates the effect of sublethal cyanide primarily on the release of exogenous yolk, rather than its induction. This assumption is based on the hepatosomatic indices which indicated in estradiol-induced fish, that maximum mean HSI levels were attained on day 7 of the experiment (Table 8). The peak HSI value suggests that most of the yolk precursors has been formed in the liver prior to cyanide exposure.

These results indicate that sublethal exposure of estradiol-induced immature rainbow trout to 0.02 mg/L HCN causes significantly higher serum levels of

exogenous yolk on day 12, which subsequently decline to mean levels that are significantly lower than the mean levels observed for estradiol-induced rainbow trout.

In order to determine the overall amount of exogenous yolk in the serum throughout the seventeen-day experimental period, the areas under the curves for changes in mean HSI, serum calcium, and serum phosphoprotein phosphorus levels were calculated for estradiol-induced and estradiol-induced cyanide-exposed rainbow trout. While the area under the curve for changes in mean serum calcium and serum phosphoprotein phosphorus levels over the seventeen-day experimental period in the estradiol-induced cyanide-exposed group was higher than that of the estradiol-induced group (Table 8), the differences between the two groups may be a result of serum calcium and serum phosphoprotein phosphorus in the estradiol-induced rainbow trout reaching peak levels one day later; i.e. day 18. Extrapolating the mean serum calcium and serum phosphoprotein phosphorus levels from day 17 to day 18 for estradiol-induced and estradiol-induced cyanide-exposed rainbow trout (Figure 11), the areas under the curve for both groups

are similar, with calculated values of 295.0 and 296.2 units for calcium in the estradiol-induced and estradiol-induced cyanide-exposed fish, and values of 2476.1 and 2296.1 units for phosphoprotein phosphorus, respectively. This suggests that the amount of exogenous yolk present in the serum over the 17-day experimental period is not altered by cyanide. A possible explanation for these results is that the exogenous yolk may be released more rapidly from the liver. This may be the result of an increase in thyroid activity in cyanide-exposed rainbow trout. Such an increase in thyroid activity may stimulate the liver in some way to cause a faster release of exogenous yolk.

While there have not been any studies to date in the literature which deal with the effects of sublethal cyanide exposure on thyroid activity in fish, there are some reports which discuss the effects of potassium thiocyanate (KSCN) on the fish thyroidal system. This is particularly important in studying cyanide toxicity, since it has been demonstrated that in mammals, cyanide is converted to thiocyanate ( $\text{SCN}^-$ ) by the liver enzyme rhodanese (Westley, 1981). Although a similar mechanism has yet

to be demonstrated in fish; Raymond and Leduc (1983) have reported a significant increase in blood plasma thiocyanate levels of rainbow trout exposed to 0.01, 0.02, and 0.03 mg/L HCN for twenty days. This suggests that in fish, as in mammals, cyanide is converted to thiocyanate. The effects of potassium thiocyanate on the fish thyroidal system have been demonstrated by Eales and Shostak (1983), who reported that intraperitoneal injections of KSCN in rainbow trout at a dosage of 1.5 mg/100 g/day for 8 days resulted in a decrease in iodine uptake by the thyroid gland. However, at a dosage of 1 mg/100 g/day they reported a significant increase in the serum levels of thyroxin (T4) and triiodothyronine (T3), as well as an increase in the ratio of T3:T4 from 1:6, to 1:3 suggesting an increase in circulating levels of metabolically active thyroid hormones. Leduc et al. (1982) reported that sublethal exposure to cyanide results in more rapid general metabolism in fish and suggest that this phenomenon may be a result of a slight hyperthyroidal condition existing in the cyanide-exposed fish. While there does not exist any evidence in the literature suggesting that the thyroid hormones stimulate the release of exogenous



yolk from the liver, it is possible that a general increase in thyroid hormones could be responsible for initiating a faster release of exogenous yolk from the liver of cyanide-exposed rainbow trout.

A second possible explanation for the faster release of exogenous yolk, which is a more specific effect, is that cyanide may be inhibiting the production of estrogen binding proteins. This would result in elevated circulating levels of free estradiol, since there would not be any proteins to bind the estradiol upon absorption. This would in turn result in more extensive binding of the estradiol to its hepatic receptor; i.e. increase its efficacy (Gilman et al., 1980). Consequently, the mechanism responsible for the synthesis and release of exogenous yolk would be speeded up. In mammals, estrogens are bound to the steroid hormone-binding globulin (SHBG) and only 1-3 % of total estrogens circulates free (unbound). Advantages of protein binding of hormones include increased half-life of the hormone, solubility and therefore transport of the hormone in circulation, and prevention of large fluctuations in hormone levels (Tepperman, 1980). A

similar system in fish has been reported by Forstier and Breton (1975), who demonstrated the presence of two, steroid binding systems in sexually mature female rainbow trout using saturation analysis and competitive binding assays. They observed that one system is a "sex binding protein" with a high affinity for estradiol-17 $\beta$  and testosterone. The second system, a transcortin type system, has a high affinity for corticosteroids, progesterone, estradiol-17 $\beta$ , and testosterone.

A third possible explanation is that the clearance rate of exogenous yolk from the serum may be altered by cyanide. This could result in an accumulation of exogenous yolk in the serum and therefore account for the higher serum levels of exogenous yolk. While the present model does not take into account the clearance rate of exogenous yolk, it does not appear likely that cyanide would inhibit the breakdown of exogenous yolk. There are three major physiological compartments which are prevalent in this study. The first is responsible for producing the yolk, i.e. the liver; the second compartment is where the yolk is released, i.e. the serum; and the third is the excretory pathway which has not been defined in estradiol-induced immature fish.

(Figure 1). In Figure 11 both mean serum calcium and mean serum phosphoprotein phosphorus levels in estradiol-induced cyanide-exposed rainbow trout peaked on day 12 with significantly higher serum levels than in estradiol-induced fish. However, the mean HSI were lower in the estradiol-induced cyanide-exposed group as compared to the estradiol-induced group. An identical pattern was also observed in Figure 10 in which serum levels peaked earlier in estradiol-induced cyanide-exposed rainbow trout than in estradiol-induced fish while mean HSI were lower in the estradiol-induced cyanide-exposed rainbow trout. In Figure 8 in which there were no significant differences in serum levels between estradiol-induced cyanide-exposed and estradiol-induced rainbow trout there were also no significant differences in mean HSI between the two groups. If sublethal exposure to cyanide were to decrease the clearance rate of exogenous yolk from the serum then no effect on HSI would be expected since this compartment is not dependant upon yolk concentrations in the serum. However, the serum compartment is directly related to the liver since it is the liver that releases the yolk into the serum. The fact that all three physiological compartments are interdependant on each

other and because of the lower HSI in estradiol-induced cyanide-exposed fish being associated with earlier peaks in serum calcium and serum phosphoprotein phosphorus levels, the results appear to suggest a faster release of exogenous from the liver as opposed to a slower clearance rate of the exogenous yolk.

Non-induced cyanide-exposed rainbow trout did not demonstrate any significant differences ( $P < 0.05$ ) in HSI, serum calcium, and phosphoprotein phosphorus levels from the untreated controls for the experiment conducted in January. When the experiment was performed in March, the HSI values of the non-induced cyanide-exposed rainbow trout were significantly ( $P > 0.05$ ) lower than those of the untreated controls on days 12 and 17, but returned to control values by day 25. Serum calcium and phosphoprotein phosphorus levels were not significantly different in this experiment. The lower HSI values may be a result of an increase in sensitivity of the fish to cyanide

McGeachy and Leduc (1983) found that the sensitivity of rainbow trout to cyanide begins to decrease in March, according to 96 hr LC50 measurements. It is therefore possible that immature rainbow trout undergo a change in seasonal hormonal cycles, rendering them more sensitive to sublethal cyanide exposure at this time of the year.

One purpose of this study was to develop an experimental model which could be used to test the effects and/or mechanisms of toxicants on exogenous yolk production. A fundamental problem in studying exogenous yolk production in sexually mature female fish is that it takes place for only a short period of time during the annual cycle of oogenesis. van Bohemen and Lambert (1981) have observed that exogenous yolk, as measured by vitellogenin, is produced annually from September to February in sexually mature vitellogenic female rainbow trout with peak levels occurring in November. In addition, a large laboratory fish population is needed in order to obtain a meaningful sample size of vitellogenic females. It is thus significant that this experimental model can be used throughout the year.

using both immature male and female fish. The results of this study indicate that serum calcium and phosphoprotein phosphorus levels in estradiol-induced and estradiol-induced cyanide-exposed immature rainbow trout were not significantly ( $P < 0.05$ ) different with respect to the response of males and females within each group. This indicates that both males and females can be used in this experimental model. These results confirm previous studies by Campbell and Idler (1980), who did not find any significant differences in vitellogenin levels between immature male and female rainbow trout which had been given four estradiol injections over a twelve-day experimental period.

The third series of experiments dealt with the effects of sublethal cyanide exposure on the induction of exogenous yolk synthesis. This consisted of pre-exposing rainbow trout for thirteen days to cyanide (0.02 mg/L), prior to estradiol injections. The first estradiol injection was administered on day 13 and the fish were sampled on day 21. The results suggest that exogenous yolk production in the cyanide pre-exposed rainbow trout was induced by the

estradiol injections. The HSI values of the estradiol-induced cyanide-exposed rainbow trout were lower than those of the estradiol-induced group, while being higher than mean control values. Serum calcium and phosphoprotein phosphorus levels for the estradiol-induced cyanide-exposed group were not significantly different from the estradiol-induced group but both groups were higher than control rainbow trout thereby indicating exogenous yolk production was induced in both groups. These results suggest that cyanide does not alter the process by which estradiol induces the liver to produce exogenous yolk.

The acute effect of cyanide as an inhibitor of mitochondrial respiration (Solomonson, 1981) has generated a widespread belief that cyanide toxicity is associated with general metabolic inhibition. While the acute toxicity of cyanide can be related specifically to respiratory inhibition by  $CN^-$ , the pathological effects of sublethal cyanide exposure in fish may be due to a different mechanism of toxicity, such as the accumulation of  $SCN^-$  in circulation (Leduc, 1981). While exposure to sublethal cyanide

concentrations of 0.01 and 0.02 mg/L has been reported to significantly decrease yolk deposition in rainbow trout (Lesniak and Ruby, 1982), the results obtained in this study would suggest that the rate of production and release of exogenous yolk is enhanced by cyanide exposure. The resultant effects of a faster release of exogenous yolk on secondary yolk deposition in the oocytes of naturally reproducing female fish may be responsible for a decrease in secondary yolk deposition. The uptake of exogenous yolk from the serum by the developing oocyte is stimulated by a gonadotropin released from the anterior pituitary (Ng and Idler, 1979). Bromage et al. (1982) reported that the annual cycle of gonadotropins peaked twice a year in sexually mature female rainbow trout. While Bromage and his co-workers did not discriminate between the different gonadotropins, i.e. those involved in stimulating endogenous yolk production and those involved in initiating exogenous yolk uptake, their study indicates a first gonadotropin peak in July at the onset of endogenous yolk production and a more prominent peak in February during the final stages of exogenous yolk uptake. Furthermore they found that



circulating levels of exogenous yolk, as measured by serum calcium and serum phosphoprotein phosphorus, peaked in October. Following this peak in exogenous yolk there occurred a decrease in exogenous yolk from the serum which coincides with the increase in gonadotropins, thereby suggesting a possible uptake of exogenous yolk from the serum by the developing oocyte. If the rate of release of exogenous yolk was enhanced as the present study would suggest, then it is possible that the levels of exogenous yolk in the serum would become out of phase with the release of gonadotropins and thus the exogenous yolk may not be incorporated into the developing oocyte. This in turn would result in a decrease in secondary yolk deposition.

This study has raised many new questions on the actual mechanism by which sublethal cyanide toxicity inhibits production. Seasonal studies dealing with the effects of a faster release of exogenous yolk on yolk deposition in sexually mature fish are needed in order to understand the consequences of an increase in the rate at which exogenous yolk is released from the liver under sublethal cyanide exposure. Further

work is also needed to study the mechanism of release of exogenous yolk from the liver in both sexually mature female fish undergoing natural vitellogenesis and in estradiol-induced immature fish. Such a study could determine which physiological factors are stimulated by cyanide which result in a faster release of exogenous yolk from the liver. While the experimental model described in this study is the first of its kind intended for toxicology, it serves a purpose in preliminary experiments aimed at determining toxic mechanisms which inhibit female reproductive function in fish. This model is the first step in the development of a more complex and complete model designed to completely evaluate the mechanism of yolk production under toxic stress. In order to achieve this further research is required to develop hormonal implants which could be used not only to stimulate exogenous yolk production but also to stimulate yolk uptake and endogenous yolk synthesis. This would result in a model which could completely evaluate the process of vitellogenesis under toxic stress.

The effects of environmental toxicants on

reproductive processes in fish have received greater attention as bodies of fresh water are becoming more polluted and fish populations<sup>o</sup> are steadily declining. To date, few studies have tried to determine the mechanisms by which fish undergo reproductive failure. This study has provided a new approach in developing an experimental model aimed at understanding the mechanisms by which aquatic toxicants inhibit reproductive cycles in female fish. It is hoped that this model will serve a purpose in understanding mechanisms of toxicity, and that it can be used in the regulation of safe levels of industrial effluents, as a means of protecting the aquatic ecosystem.

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APPENDIX

Appendix 1: Mean serum calcium, serum phosphoprotein phosphorus and serum vitellogenin levels in rainbow trout injected with 5 mg/kg of body weight of estradiol on days 0 and 3 of a 12-day experiment in which exposure to 0.02 mg/L HCN started on day 0. Fish were sampled on day 12. Experiments were performed at  $12.0 \pm 1.0^\circ\text{C}$  during October, 1983. Serum vitellogenin levels were determined by Dr. D.R. Idler (Dept. of Biological Sciences, Memorial University of Newfoundland).  
Note: E2 = estradiol-induced; E2 + HCN = estradiol-induced cyanide-exposed.

Day	Group	Serum Calcium (mg%)	Serum Phosphoprotein Phosphorus (ug/ml)	Serum Vitellogenin (mg/ml)
12	Control (n=2)	9.3 $\pm$ 1.3	49.1 $\pm$ 9.4	0.31†
	E2 (n=3)	20.4 $\pm$ 2.9	213.5 $\pm$ 4.5	6.21†
	E2 + HCN(n=4)	26.0 $\pm$ 4.4	277.0 $\pm$ 69.8	37.9 $\pm$ 13.6

† Pooled serum samples for vitellogenin analysis.

Appendix 2: Areas under the curve for estradiol-induced and estradiol-induced cyanide-exposed rainbow trout which were injected with estradiol on days 0 and 3 of a 17-day experimental period in which cyanide exposure started on day 0. The areas were calculated according to Simpson's Rule (Apple Statpak) with the x-axis ranging from day 0 to 17 (Figure 10).

Area Under The Curve	E2-induced	E2-induced + HCN
HSI	38.3	32.2
Serum Calcium	235.7	265.2
Serum Phospho-protein Phosphorus	2350.2	3299.1