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CHRONIC EFFECTS OF CYANIDE ON REPRODUCTION AND DEVELOPMENT OF AMERICAN FLAGFISH, *JORDANELLA FLORIDAE*

Samuel Kwong-ho Cheng

A Thesis

in

The Department

of

Biological Sciences

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for the degree of Master of Science at
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ABSTRACT

SAMUEL KWONG-HO CHENG

CHRONIC EFFECTS OF CYANIDE ON THE REPRODUCTION AND DEVELOPMENT OF AMERICAN FLAGFISH (*Jordanella floridae*)

Newly fertilized eggs of American flagfish, Jordanella floridae, were subjected to 0.065, 0.075, 0.087, and 0.15 mg/L HCN from the time of fertilization until hatching in continuously renewed water at 25±0.5°C. Chronic effects of hydrogen cyanide were measured in terms of hatching time, hatching success, fry survival, growth, yolk utilization efficiency, and incidence of abnormalities. Hatching time of embryos incubated in 0.065, 0.075, and 0.087 mg/L HCN was increased by approximately 23% as compared to the controls; while at the highest concentration tested, (0.15 mg/L HCN), it was 89% longer than the controls. Hatching success increased with decreasing concentrations. Values of 85.6%, 67.5%, 56%, and 3% were recorded at 0:065, 0.075, 0.087, and 0.15 mg/L HCN respectively when compared to the control value of 89%.

Yolk conversion efficiency into body tissue in cyanide exposed larvae was reduced to 14.89%, 9.98%, and 4.4% at 0.065, 0.075, and 0.087 mg/L HCN compared with the control value of 22.7%.

When cyanide was removed upon hatching, a faster growth rate was observed at 14 and 28 days post-hatching in all cyanide treated larvae. Cyanide-induced anomalies included microphthalmia and monophthalmia as well as body flexures. Measurements of pituitary gland of larvae indicated significant reduction in both mean length and width

at all cyanide concentrations tested.

Exposure of eggs from fertilization to hatching lowered fecundity levels when females reached sexual maturity. Exposure to hydrogen cyanide prior to hatching and again for five days during juvenile development further reduced fecundity at sexual maturity. It suggests that this additional reduction in magnitude may be the result of damage to the developing oogonia during juvenile development. Fecundity of females exposed to cyanide from fertilization to hatching and again for five days at sexual maturity does not significantly differ from a single exposure during the embryonic development and suggests that a short term exposure of five days during sexual maturity has no effect upon developing eggs within the ovary of females. Delayed spawning, however, and shortened estrous cycle were evident and are believed to relate to damage imposed upon the pituitary gland during embryonic development. The results emphasized the importance of assessing the effect of intermittent exposure to a toxicant upon various life stages of an organism.

Acute toxicity of hydrogen cyanide was determined for 24, 48, 72, and 96-hour old fry. 96-hr. LC50 were 0.42, 0.44, 0.65, and 0.85 mg/L HCN for 24, 48, 72, and 96-hour old fry respectively, suggesting that 24-hour old fry represent a most critical time in the early life history of flagfish. Resistance of fry to hydrogen cyanide increased steadily with age.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to all who have helped me to accomplish this project. In particular, I thank my fellow graduate students and friends: J. Lesniak, D. Galley, N-J. Hibberd, T. Kovacs, M. Gaudet, I. McCracken, and W. Banas for their advice and encouragement.

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INTRODUCTION

Stress beyond the normal tolerance limits of an organism, imposed during the critical reproductive processes, can ultimately lead to the decline or disappearance of a species in nature. Detrimental effects produced by stress from water pollutants are usually manifested physiologically or morphologically in an organism prior to the loss of an entire population from the ecosystem.

Cyanide, long known as a respiratory depressant, is among the most hazardous water pollutants associated with the steel and heavy metal industry. In aquatic ecosystems, free cyanide concentrations may vary from 0.1 mg/L to as high as 30 mg/L HCN (Bērubē and Gilbert, 1971). The selection of this contaminant is particularly relevant to Canadian waterways since cyanide is a major industrial waste in the north, as a result of an active mining industry.

This laboratory study is designed to elucidate the sublethal effects of hydrogen cyanide on the reproductive success and development of the American flagfish, Jordanella floridae. Several parameters have been selected for study including egg incubation time, hatching success, fry survival, yolk utilization efficiency in post-hatched larvae, larval growth, incidence of larval teratogenic defects, and a life study of one generation of flagfish to evaluate the effects of cyanide on embryonic development and reproductive capabilities, following cyanide exposure at the embryonic, juvenile, and adult stages.

Chronic cyanide effects upon the pre-spawning gametes in

the maturing fish gonads have not been thoroughly investigated. Only two studies pertinent to this subject are documented. Ruby and Dixon (1974) reported that the mitotic activity of the developing spermatogonia in 18-month rainbow trout, Salmo gairdneri, diminished significantly following exposure to hydrogen cyanide concentrations of 0.01 mg/L HCN for twenty days at 12.5°C. At the same concentrations, they noted cyanide inhibited metaphase, preventing further maturation of developing germ cells. This could ultimately lead to reduced sperm production. The effects on chronic cyanide on rainbow trout ovarian development were subsequently investigated by Lesniak (1977). Exposure of maturing female rainbow trout to 0.01 mg/L HCN for twenty days produced increased frequencies of atresia at all developmental stages, indicating that cyanide may decrease final fecundity in females. Morphological changes in developing oocytes suggested alteration in protein and carbohydrate synthesis along with disturbances in primary and secondary yolk deposition. The overall effects indicated that cyanide selectively retarded growth of oocytes and delayed the development. These previous investigations demonstrated that hydrogen cyanide impairs developing pre-spawned gametes in fish, however, they provide no information of the effects of cyanide on early embryonic development in post-spawned fish eggs.

The early life phase of fish is particularly sensitive to both chemical and physical changes in an aquatic ecosystem (Rosenthal and Alderdice, 1976). Fluctuation in these conditions can be deleterious to development at this stage. Early physiological events such as hatching

time, hatching success, and fry survival have been reported to be altered by a change in salinity, temperature, and the presence of cadmium and ammonia (Blaxter, 1969, Alderdice and Velsen, 1971, Rosenthal and Sparling, 1971, and Burkhalter and Kaya, 1977).

Two studies specifically related to cyanide exposure and developing embryos have also been reported. Crawford and Wilde (1966a) incubated Fundulus eggs at all developmental stages in high cyanide concentrations and demonstrated that cyanide is a selective inhibitor of fish embryogenesis following gastrulation. Leduc (1978) reported that hydrogen cyanide concentrations at sublethal levels can be deleterious to eggs and juvenile fish. He incubated newly fertilized eggs of Atlantic salmon in concentrations of cyanide ranging from 0.01 to 0.1 mg/L HCN until hatching, and reported that cyanide reduced hatching success by 15-40% depending on the concentrations. Incidence of gross abnormalities in cyanide treated fish was high even in concentration as low as 0.01 mg/L HCN. Although it is evident from these studies that cyanide is detrimental to the early life stages in fish, yet all previous investigations were conducted with cold water fish. Reports of chronic cyanide toxicity on hatching time, hatching success, and survival in warm water species remain undocumented. It is thus one purpose of this investigation to evaluate the immediate impact of sublethal cyanide on hatching time, hatching success, and survival in flagfish embryos, a warm water species.

Physiological events of early life phases in organisms are interlinked with one another. Defects at one phase may manifest itself at a later phase in development. It is therefore crucial to

to study the effects of cyanide exposure from spawning to hatching on the physiological events that follow hatching. The efficiency of yolk utilization is often regarded as a valid parameter to measure the growth of post-hatched larvae from fish incubated under different environmental conditons (Jones, 1972). Incubation temperature has been shown to affect the yolk conversion efficiency in several marine and freshwater fishes (Blaxter and Hempel, 1966, Marr, 1966, and Jones, 1972). Cyanide has been demonstrated to reduce yolk conversion efficiency in Atlantic salmon fry (Leduc, 1978). Since there is no previous report of the effects of sublethal hydrogen cyanide on yolk conversion efficiency in a warm water species, it was decided to investigate this parameter in flagfish, a species which requires a high temperature to complete embryonic development.

Growth of sac-fry is dependent on its yolk conversion efficiency (Jones, 1972). Hence, if the presence of hydrogen cyanide affects the yolk conversion efficiency, alteration of growth rate in fry can be expected. Growth of fish larvae under hydrogen cyanide influence has only received limited attention (Doudoroff, 1976). Only three studies have been documented (Leduc,1966b, Lind,et al, 1977, and Koenst, et al, 1977). All these experiments were conducted under continuous toxicant exposure. Results of these investigations demonstrated that cyanide at 0.06 mg/L HCN induced juvenile cichlid (Cichlasoma bimaculatum) to grow faster (Leduc,1966b). Lind, et al, (1977), however, showed that HCN at 0.06 mg/L did not affect the growth in fathead minnow. Nonetheless, Koenst, et al, (1977) reported that hydrogen cyanide concentration at 0.03 mg/L and above affected growth by retardation in

juvenile brook trout. The present study on growth of sac-fry differs from the previous investigations since flagfish eggs were exposed to cyanide until hatching and did not receive continuous toxicant exposure. The results of growth in sac-fry will therefore, reflect damage which occurs following cyanide exposure from the time of spawning to hatching only.

Malformations in developing fish embryos have been produced under the influence of both physical and chemical agents including low temperature (Stockard, 1921, Briggs and Wilson, 1959), an azo dye, trypan blue in developing zebrafish (Battle and Laale, 1960). Abnormalities in embryos of Fundulus (Oppenhemiar, 1950), Oryzias (Watermann, 1939, and Ishida, 1951), and Brachydanio (Anderson and Battle, 1967) have been reported after exposure to various drugs. Recently, the effects of hydrogen cyanide have been reported to induce anomalies in Atlantic salmon embryos (Leduc, 1978). No work has yet been done on the teratogenicity of cyanide in warm water fish larvae. It is the purpose of this study to investigate this aspect, using flagfish as the test species.

Although the immediate effects of sublethal hydrogen cyanide on the physiological events of the early life phases of fish are important, yet they do not reflect the overall long term impairment which a toxicant may have on the reproductive capabilities of an adult fish and the subsequent embryonic development of the next generation. Sprague (1976) stressed the importance of conducting chronic toxicity studies to evaluate the effects of toxic agents over several generations of a test organism. Current literature reviews indicate that only two studies of the effects of cyanide chronic poisoning on the life cycle

of fish have been performed. In the first study, Lind, et al, (1977) investigated the long term effects of continuous hydrogen cyanide exposure at concentrations ranging from 0.005 to 0.1 mg/L HCN in fathead minnow, Pimephales promelas, at 25°C from hatch into the second generation juvenile stage. Mortality within the first month of exposure was high in both the parent and the F_1 offsprings. Fecundity of the females and egg hatchability were significantly reduced relative to the controls. In the second study, Koenst, et al, (1977), observing the chronic effects of hydrogen cyanide on brook trout over two generations, demonstrated a delay in spawning time of fish exposed to cyanide at concentrations of 0.01 to 0.075 mg/L HCN. Reduction in the number of spawnings and the number of eggs laid by the F_1 generation females were noticed in fish exposed to the same concentrations of cyanide. No fertile eggs were found in the spawning females exposed to 0.065 and 0.075 mg/L HCN. Although cyanide seemed to have no effect on the hatchability of eggs exposed, the survival of larvae was adversely affected at 0.05 mg/L HCN.

Previous investigations testing the chronic effects of cyanide were performed under a continuous exposure system when test fish were exposed to toxicants throughout the entire experimental period. In nature, however, the occurrence of toxicants can be inconsistent. Thus, the second purpose of this study was to investigate the intermittent sublethal effects of hydrogen cyanide throughout one generation in flagfish. Periodic introductions of cyanide into the water were carried out both singly and in combination during the embryonic, juvenile, and adult stages. The early embryonic and larval physiological events

such as the hatching time of eggs, hatching success, and survival of fry as influenced by cyanide were selected as parameters for embryonic studies, while effects on juveniles and adults were assessed through fecundity studies.

The purpose of the present study is thus, twofold. It will investigate and compare the relative sensitivities of the immediate sublethal effects of hydrogen cyanide at several stages of embryonic development in flagfish embryos, a warm water species. Secondly, it will measure the effects of intermittent cyanide exposure during embryonic, juvenile, and adult stages of a complete generation along with the subsequent effects on the embryonic development of the following generation.

MATERIAL, APPARATUS, AND METHODS

Material

Stock Fish

The original stock of American flagfish, Jordanella floridae, was purchased from the Hartz Mountain Pet Supplies Ltd. It consisted of twenty males and forty females with an average weight and length of 3.5 grams and 2.5 cm. respectively. All subsequent generations of the test fish were reared from this stock in the laboratory.

Upon arrival at the laboratory, the stock fish were maintained in 67-liter all glass aquaria, with a holding density of 15 fish per tank. Approximately 20% of the tank water was renewed daily. Each aquarium was equipped with a filter containing glasswool and charcoal to facilitate removal of fish metabolites and suspended detritus. The water temperature of the tanks was maintained constantly at 25±0.5°C. Fish were fed three times daily on a diet of frozen brine shrimp, Artemia, supplemented by an additional daily feeding of Tetra Growth food.

Apparatus

Test Tanks

The entire experimental apparatus for this study was comprised of ten all-glass test tanks which measured 60 cm. long, 30 cm. wide, and 30 cm. deep (Figure 1.). Adjustable flowmeters (Manostat Corp., New York.) were employed to maintain the flow of water to each tank at a rate of 1 liter per minute. Water in each tank was continuously drained by a $1\frac{3}{4}$ cm. diameter standpipe producing a volume of 67 liters

in each tank at any given time and allowing a 99% replacement of freshwater in 4½ hours (Sprague, 1973). Mariotte bottles (Leduc, 1966a) were used to meter the toxicant into the test tanks.

Light

The experimental tanks were illuminated by 40-watt fluorescent lights (Lifeline, Model F-40, warm white, Sylvania Can.) which were positioned parallel to and three feet above the middle rear position of the tanks to provide an even intensity of light on all tanks. The lighting system was controlled by a time-switch and was further modified with dawn-dusk simulation apparatus (Dawson and Drummond, 1970). A sixteen hours photoperiod was maintained throughout the entire experimental period. A black opaque plastic sheet was used to enclose the apparatus so as to minimize the effects of visual disturbance and undesirable light sources emitted from the adjacent experimental apparatus.

Water Supply

Experimental tanks were supplied with City of Montreal water heated to 25±0.5 °C following removal of chlorine by activated charcoal dechlorinators. Water was delivered to the apparatus via plastic (PVC) tubing. Oxygen supersaturation during winter was prevented by immersion of air-stones into the headtank. The water was delivered from the headtank through an air stripping column which effectively reduced supersaturation to the minimum. Routine maintainence of the apparatus involved daily cleaning of the tanks and timing of the flowrates.

Chemical Analysis

In the course of the experiment, analysis of cyanide

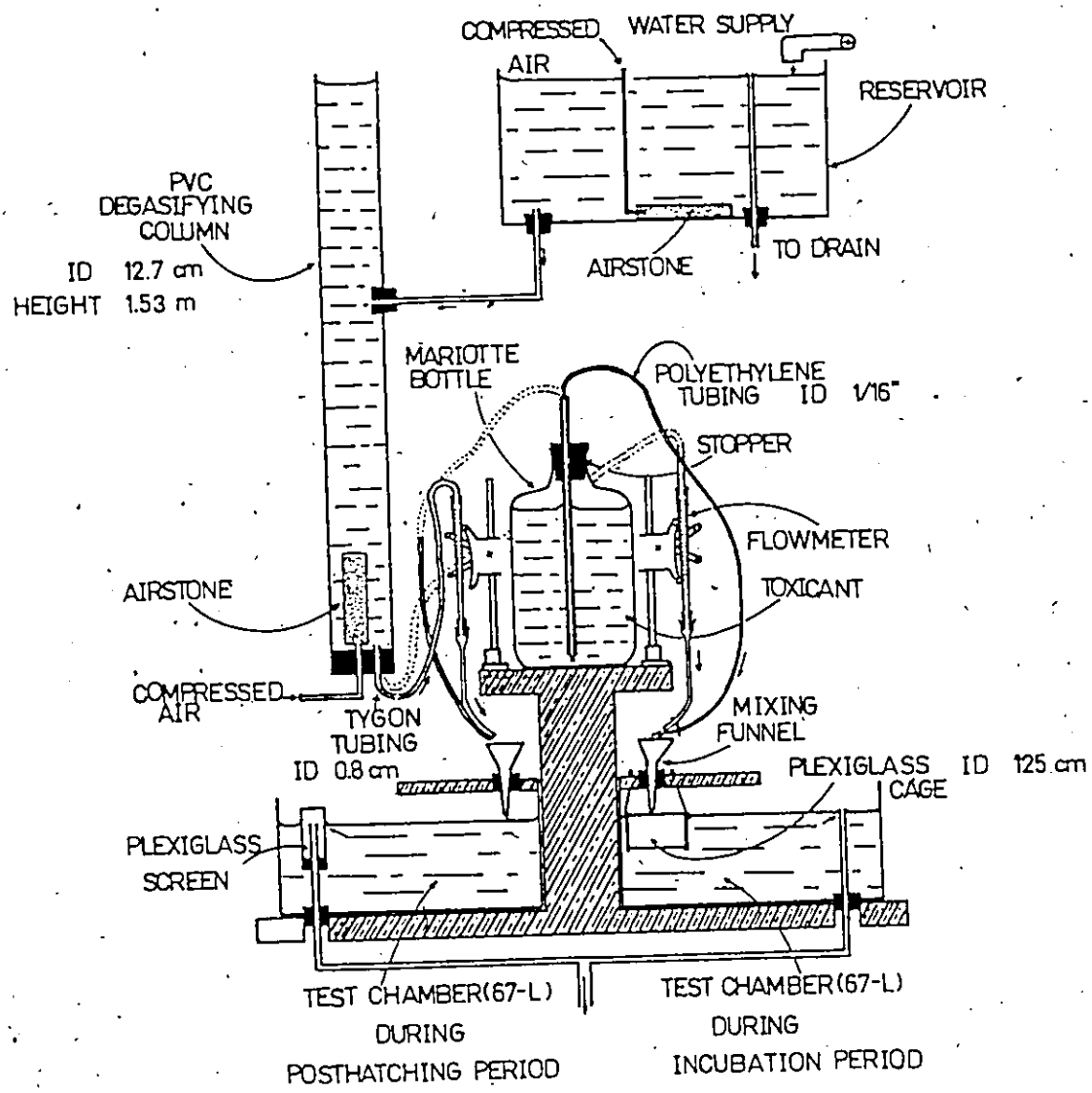


FIGURE 1. Schematic Drawing of the Experimental Apparatus Used for the Continuous Exposure of American Flagfish Eggs and Fry to Sublethal Concentrations of Hydrogen Cyanide

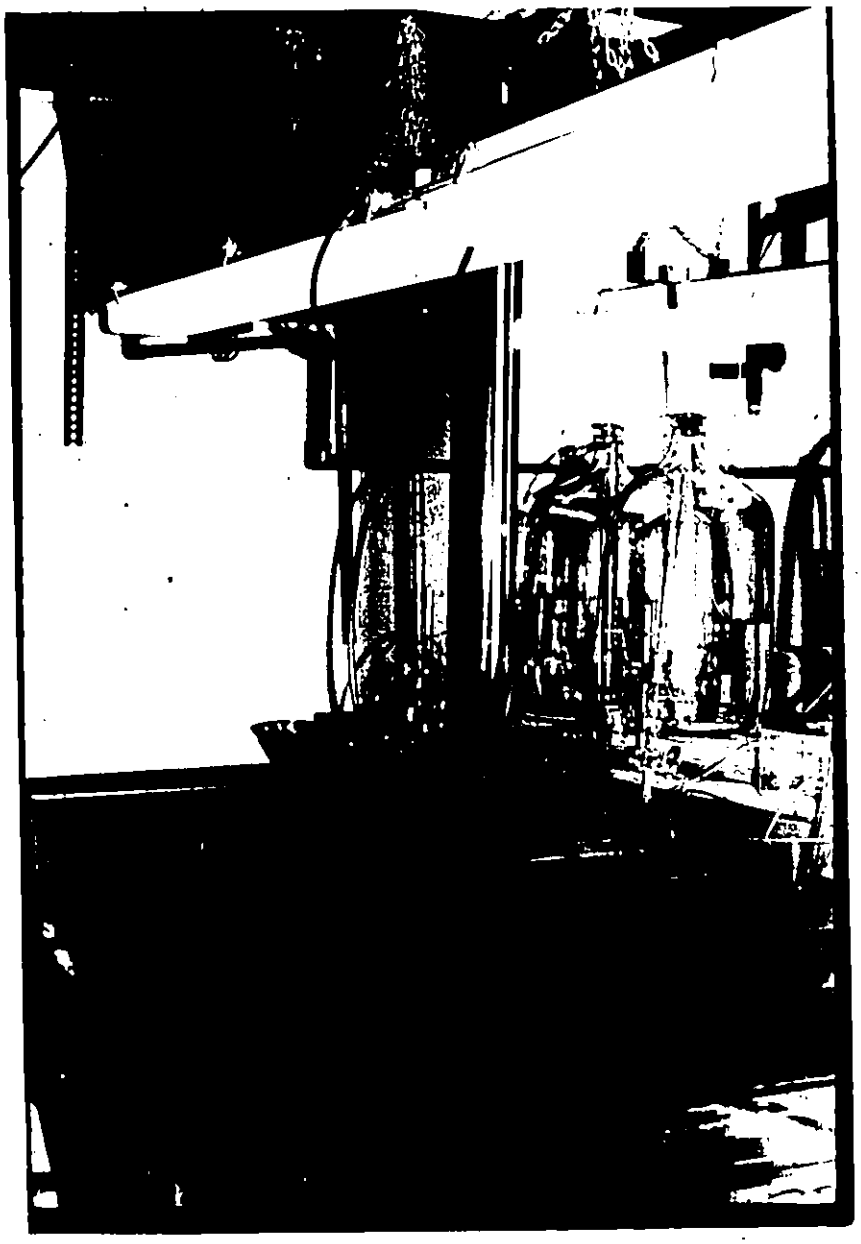


Plate 1. The experimental apparatus with Mariotte bottles, dilution funnels, and aquaria.

concetrations in the water of experimental tanks was determined daily by the method outlined by Lambert, et al., (1975). Strength of cyanide was estimated from a standard curve obtained using a spectrophotometer (Spectronic 70, Bausch and Lomb) set at 575 um wavelength following a twelve minute incubation period of water samples at 25° C. Cyanide levels in experimental tanks were maintained within predicted concentrations. Infrequently, fluctuation within 5% of the desired values occurred and were corrected accordingly by adjusting the flow-rate of the stock solution.

On alternate days, dissolved oxygen levels in the experimental tanks were analysed by the Sodium Azide Modification of the Winkler Method (Standard Methods, 1971, p.477). Oxygen content was calculated from a standard curve outlined by Oulman and Baumann (1956). The level of oxygen saturation was maintained above 95% throughout the entire experimental period. The pH of the incoming water was constantly maintained between 7.9-8.2 pH units throughout the whole experiment. Residual chlorine was checked bi-weekly by the orthotolidine method (Standard Methods, 1971, p.117). The level of chlorine was constantly kept below 0.01 mg/L in all the experimental tanks.

CHRONIC EFFECTS OF CYANIDE ON THE REPRODUCTION AND DEVELOPMENT OF AMERICAN FLAGFISH, JORDANELLA FLORIDAE

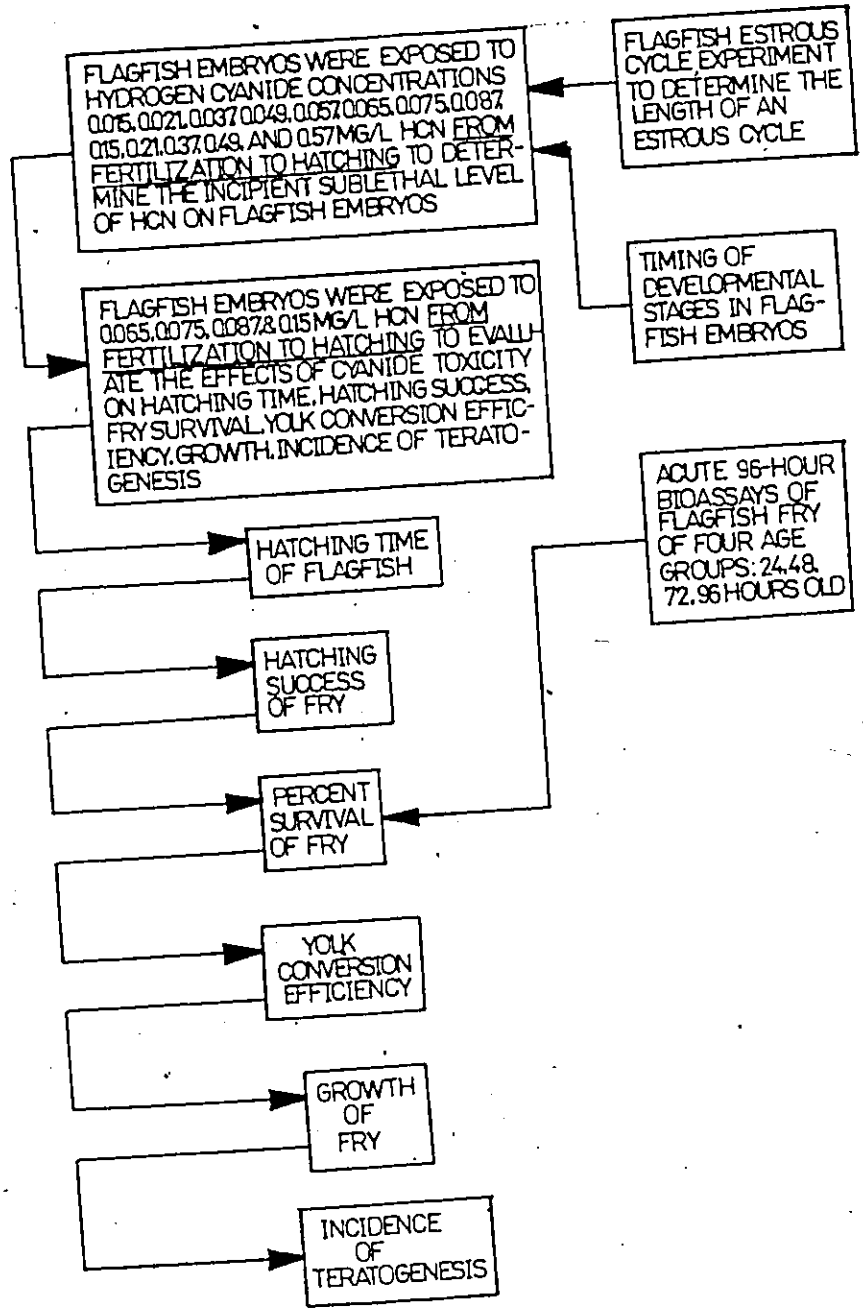


CHART 1

CHART 2
(TREATMENT 1)

EFFECTS OF CYANIDE EXPOSURE IN FLAGFISH DURING THE EMBRYONIC AND JUVENILE STAGES OF DEVELOPMENT

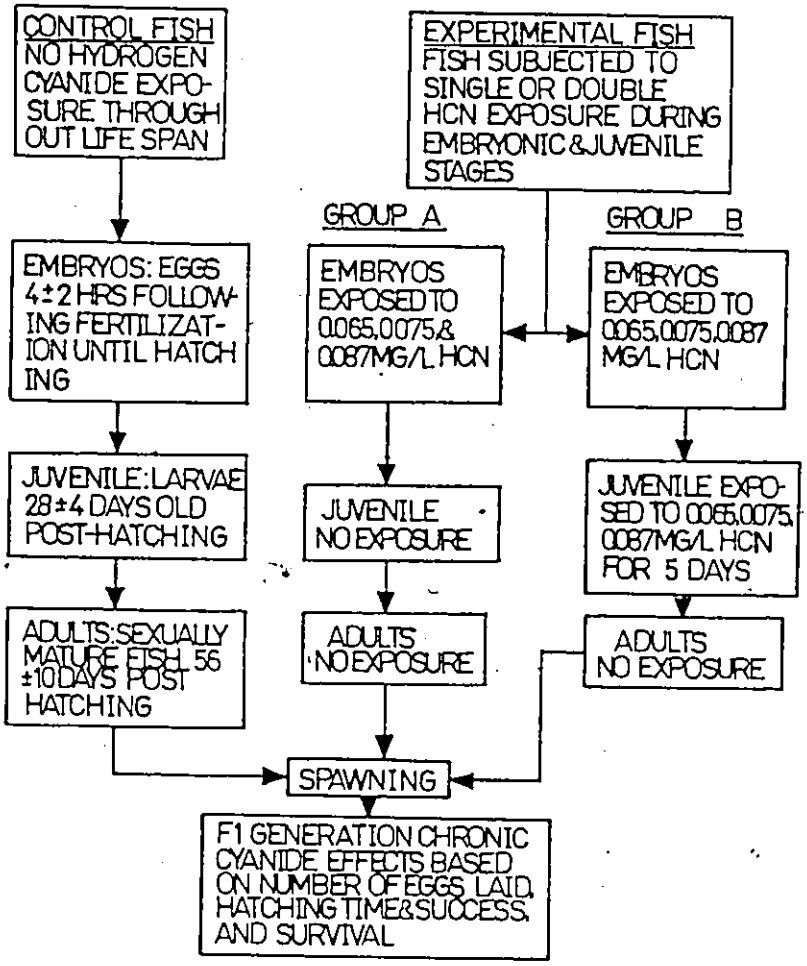
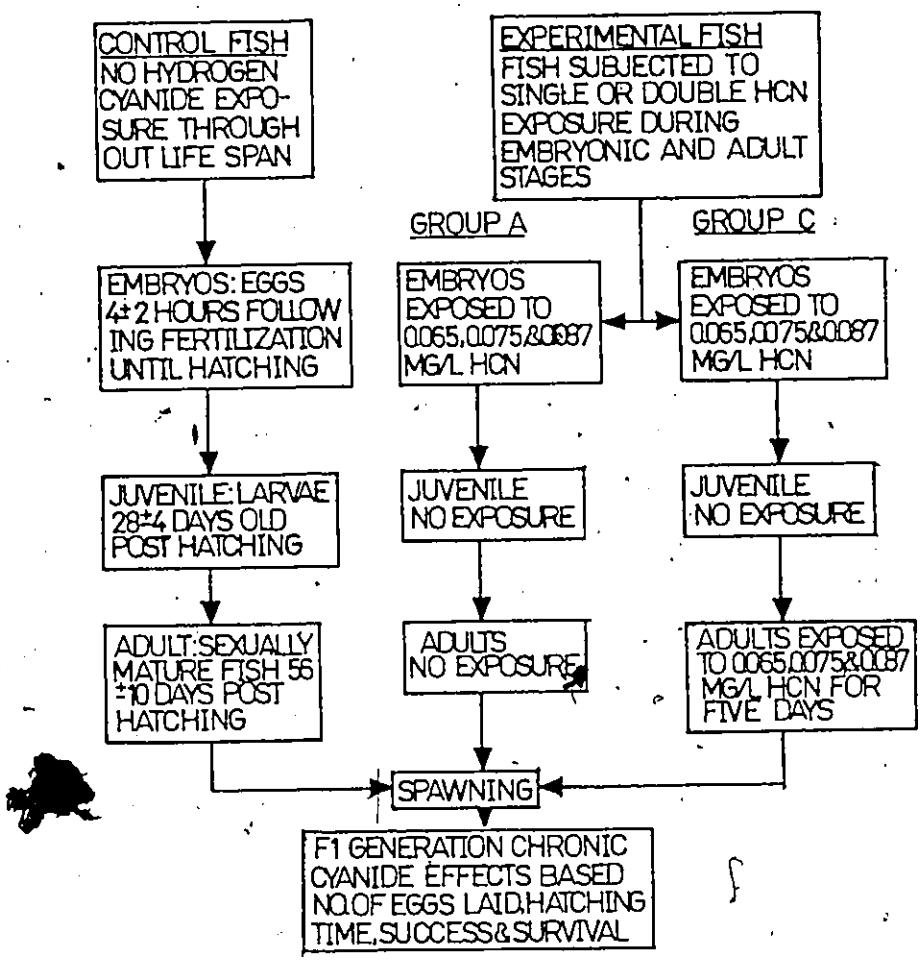


CHART 3

(TREATMENT 2)

EFFECTS OF CYANIDE EXPOSURE IN FLAGFISH DURING THE EMBRYONIC AND ADULT STAGES OF DEVELOPMENT



Methods

Spawning and Egg Selection

When sexually mature male and female flagfish were paired in a 1:1 ratio, spawning occurred on spawning mats in the breeding tanks. Eggs were obtained following the daily peak of the spawning activity which usually occurred mid to late afternoon on the 4th and 5th day following the first appearance of eggs on mats (Cheng and Ruby, unpublished data). The eggs were carefully removed and cleansed by gentle expulsion of water from a pipette. They were selected for viability on the basis of initial cleavages and utilized for subsequent experimentation. All eggs used in the experimental work were restricted to females from which at least 70% of the eggs from each spawning had begun cleavage (Anderson and Battle, 1967).

Chronic Studies

Hatchability and survival of embryos

A preliminary experiment was conducted to determine the incipient sublethal threshold of hydrogen cyanide on flagfish eggs. One hundred viable flagfish eggs were exposed from fertilization to hatching to each of a total of 13 cyanide concentrations ranging from 0.015 mg/L to 0.57 mg/L HCN (Chart 1.). The incipient sublethal threshold of hydrogen cyanide on flagfish eggs was established based upon the criteria of hatching time, hatching success, and percent survival. Four cyanide concentrations: 0.065, 0.075, 0.087, and 0.15 mg/L as HCN were selected accordingly for further investigation.

Following the preliminary experiment, a second identical

experiment was performed to evaluate the effects of chronic cyanide poisoning on flagfish. Similarly, eggs were incubated in cyanide concentrations of 0.065, 0.075, 0.087, and 0.15 mg/L HCN at 25°C. The hatching time, hatching success, and fry survival in each concentration was recorded. One hundred viable eggs (4-8 hours following fertilization) were selected and incubated in plexiglass cages which were mounted with nylon screen cloth each measuring 125 cm., dia., and 10 cm. in depth. The cages were submerged immediately below the air-water interface of each test tank and the cyanide solution was directly monitored into them, thus providing a constant flow of toxicant around the developing eggs. Suspension of cages in this manner further provided the eggs with clean water of high oxygen content.

The end point of any hatching time study was the successful hatching of 50% or more embryos at that given concentration. Expression of hatching success was based upon the number of fry that hatched compared to the initial number of eggs incubated. Fry survival data was systematically recorded for each cage, ten days following the spawning, and the percent survival of fry was expressed following the method outlined by Brungs (1971).

Yolk conversion efficiency

To investigate the efficiency of yolk conversion, twenty newly hatched flagfish larvae from both controls and cyanide treated embryos of the second experiment were removed from the test chambers. Both the body length and the yolk sac radius from these larvae were measured by means of an ocular micrometer and the volume of the yolk sac was subsequently calculated. The larvae were then released back

into their corresponding test tanks. Measurements were recorded daily, and were terminated normally on the 5th day following hatching when larval yolk sac had been completely resorbed.

The ratio of the total increase in body length to the total decrease in yolk sac volume were used to denote the growth per unit yolk resorbed (Ryland and Nichols, 1966).

Growth of sac-fry

The growth of flagfish fry following cyanide exposure from the time of fertilization until hatching was expressed as absolute growth and the relative growth in length. Absolute growth is defined as the average total size at each age whereas the relative growth is defined as percentage growth in which the increase in length in each time interval (Rounsefell and Everhart, 1966, p.313-p.314). Computation of absolute growth was based upon the length of fry at a specific age, namely: fry at hatching, fry fourteen days old, and fry twenty-eight days old. Ten larvae from each concentration were taken. Measurements of the respective larval length were made on the appropriate days. The percent growth rate of both the control and the cyanide treated fry were computed as the increase in length (fork length) of fish per day.

Incidence of abnormalities

The incidence of abnormalities induced by cyanide on the exposed embryos was examined histologically. Five embryos from each cyanide concentration were selected and fixed in either Bouin's or Davidson's fixatives, dehydrated in ethanol, embedded in paraffin (Tissuemat, m.p. 56.6 C.), serially sectioned at 8 μm, stained with Haematoxylin and Bowie's Eosin, and subsequently mounted in permount. Defects were examined at the light microscopic level.

Sections from the controls and the exposed fry were compared.

Life studies

The chronic effects of cyanide on flagfish was also tested over one generation from the time of fertilization of the first generation until hatching of fry in the second generation. Exposure to toxicant was limited to specific periods during the development, and included exposure either single or combined, during the embryonic, juvenile, and the adult stages of development (Charts 2 and 3). Eggs were selected for viability and incubated in cyanide concentrations of 0.065, 0.075, and 0.087 mg/L HCN at 25 °C on the basis of the preliminary experiments. Cyanide concentration of 0.15 mg/L was omitted in this study because it has been demonstrated in the former experiment that flagfish fry at this concentration were unable to survive more than forty eight hours following hatching. Parameters selected for study included fecundity in sexually mature females of the first generation in addition to egg hatching time, hatching success, and fry survival in embryos among the first generation offsprings. Embryos were randomly divided following hatching into controls and cyanide treated groups: A, B, and C. Group A represented individuals which had been exposed to cyanide concentrations of 0.065, 0.075, and 0.087 mg/L only during the period from fertilization to hatching. Group B were exposed to identical cyanide concentrations during the same embryonic period but received an additional five day exposure during the juvenile stage (4th week post hatching) (Chart 2.). Likewise, fish from group C were treated with the identical cyanide concentrations from fertilization to hatching but received a second exposure for five days as adults (8th

week post hatching). They were compared with results from controls and group A to determine the effects of cyanide following a double exposure during both the embryonic and adult stages (Chart 3.).

Acute Studies

Acute bioassays

Determination of the relative sensitivity of flagfish embryos to hydrogen cyanide with time was carried out according to the methods recommended by Sprague (1973). Hatched embryos from the control tanks were graded into four different age groups: 24, 48, 72, and 96 hour old fry and assayed in cyanide concentrations ranging from 0.03 to 1.0 mg/L HCN at 25 ℃. Ten fish were used in each bioassay. Mortality of the tested fish was defined as the cessation of tail movements and heart beat. The LC50 of each concentration was estimated according to Litchfield and Wilcoxon (1949).

RESULTS

Determination of the Incipient Sublethal Level of Cyanide for American Flagfish Embryos, Jordanella floridae, Following Exposure to the Toxicant from Fertilization to Hatching

Determination of the threshold sublethal level of cyanide for hatching time, hatching success, and percent survival of American flagfish was carried out in triplicates. Embryos were exposed to cyanide from the time of fertilization until hatching. Cyanide concentrations used were 0.015, 0.021, 0.037, 0.049, 0.057, 0.065, 0.075, 0.087, 0.15, 0.21, 0.37, 0.49, and 0.57 mg/L as HCN at 25 °C. Results indicated that the responses of both the mean hatching time, hatching success, and survival of flagfish embryos to cyanide were dose-dependent. Hatching time increased with increasing cyanide concentrations while both the hatching success and percent survival decreased with increasing cyanide concentrations.

Hatching Time

Hydrogen cyanide concentrations as low as 0.015 and 0.021 mg/L HCN did not cause any retardation in hatching time (Table 1.). Egg samples incubated in cyanide concentration of 0.037 mg/L HCN, however, took an average of 18 hours more to hatch than the controls. Embryos incubated in hydrogen cyanide concentrations ranging from 0.049 to 0.087 mg/L HCN had hatching time delayed 32±8 hours as compared to the controls. At 0.15 mg/L, hatching time of the treated embryos soared to 216±24 hours. The hatching time of egg samples in cyanide concentrations ranging from 0.037 to 0.15 mg/L HCN were significantly different from the corresponding control groups ($p \leq 0.05$).

Table 1. Mean hatching time, hatching success, and percent survival of 50 flagfish embryos in the determination of incipient sublethal cyanide level in a continuous flow-through system at 25°C.

Cyanide Conc. (mg/L HCN)	Mean Hatching Time (Hours)	S.D.	Mean Hatching Success (Percent)	S.D.	Mean Survival of Fry (Percent)	S.D.
0.57	>216	-	0	-	0	-
0.49	>216	-	0	-	0	-
0.37	>216	-	0	-	0	-
0.21	>216	-	0	-	0	-
0.15	216	±24	4.6	±0.3	0	-
0.087	140	± 8	55.2	±1.8	56	±6.2
0.075	140	± 8	52.0	-	68	±3.4
0.065	140	± 8	84.0	-	72	±5.7
0.057	140	± 4	85.8	±1.1	74	-
0.049	140	± 4	83.6	±2.4	76	±2.7
0.037	132	± 8	84.8	±0.7	72	±1.5
0.021	114	± 4	89.2	±1.3	76	±3.8
0.015	114	± 4	86.6	±4.0	74	±3.5
0.00	114	± 4	87.2	±1.0	76	±2.1

Hatching Success and Percent Survival

The hatching success and percent survival of fry decreased with increasing cyanide concentrations. Hatching success was deleteriously affected by hydrogen cyanide at concentrations of 0.075, 0.087, and 0.15 mg/L HCN (Table 1.). At 0.075 mg/L, hatching success was 52% which represented a reduction of approximately 40% compared to the controls in which 87.2% of the embryos hatched. Values of hatching success obtained from the cyanide treated embryos were significantly different from that of the controls ($p \leq 0.05$). Hatching success was severely retarded at 0.15 mg/L HCN. Heavy mortality of the embryos occurred in this group. Approximately 80% of the embryos died at various stages during their embryonic development. Only 5% of the total number of the exposed eggs reached the final full-term stage of development,of which less than 80% were able to initiate hatching. Hatching in this group displayed an unusual phenomenon. The hatching embryos normally ceased the hatching process as they were attempting to emerge from the egg capsule, and usually died with only the anterior or the caudal portion protruded (Plates 2 and 3.). Survival in this group did not exceed 48 hours beyond the post hatching period.

Hatching Time of American Flagfish Embryos Exposed to Sublethal Cyanide Concentrations in Continuously Renewed Water at 25 °C.

From the previous data, sublethal concentrations of hydrogen cyanide of 0.065, 0.075, 0.087, and 0.15 mg/L HCN were chosen to study the effects of cyanide on hatching time. Triplicate experiments were conducted using flagfish embryos. For the purpose of this study, hatching time was defined as the length of the incubation period from the

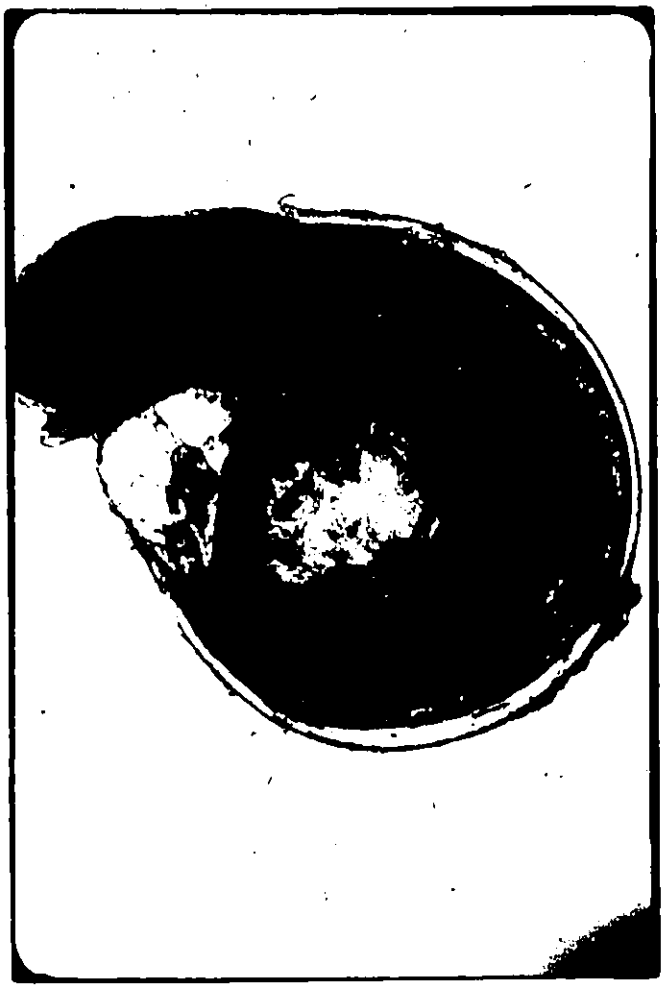


Plate 2. Partial hatching observed in flagfish embryos exposed to hydrogen cyanide at concentration of 0.15 mg/L HCN following fertilization.

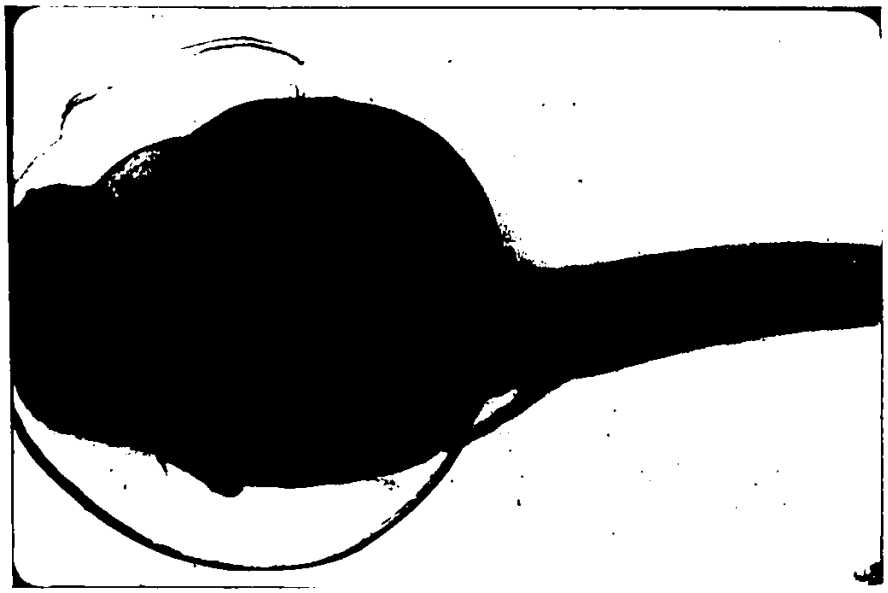


Plate 3. Partial hatching observed in flagfish embryos exposed to hydrogen cyanide at concentration of 0.15 mg/L HCN following fertilization.

time of fertilization to hatching expressed in hours.

As shown in Table 2 , the mean hatching time increased with increasing cyanide concentrations and the results agree well with the preliminary experiments (Table 1.). This indicates that the exposure of flagfish embryos to cyanide concentrations of 0.065, 0.075, 0.087, and 0.15 mg/L HCN during embryonic development of the eggs caused a marked increase in hatching time (Table 2.). Embryos exposed to 0.065 mg/L HCN hatched 140±8 hours following fertilization as compared with the controls which usually hatched 114±4 hours post fertilization. This represents a mean increase of 32±8 hours in hatching time for embryos treated with 0.065 mg/L HCN. Hatching time of embryos obtained from this concentration was significantly different from that of the control ($p \leq 0.05$). The variation, however, in incubation time was not significantly different ($p \geq 0.05$) among the treated groups.

At 0.15 mg/L HCN, the embryos exhibited the unusual phenomenon of partial hatching which was noted in the preliminary experiments. Mean hatching time in this group was 216 hours which represents a time delay of 102 hours more as compared to the controls (Table 2). Statistical analysis indicates that the hatching time between the exposed and the control embryos differs significantly ($p \leq 0.05$).

Effects of Cyanide on Hatching Success in Flagfish Embryos

Exposure of flagfish embryos to cyanide produced a decrease in hatching success with increasing concentrations of cyanide. The overall results of the effects of cyanide on hatching success of flagfish embryos are summarized in Table 3. At 0.065 mg/L HCN, the mean hatching success was 85.6% which approximately 4% lower than the controls. A more appreciable difference was noted in embryos exposed to 0.075 mg/L

Table 2. Hatching time of flagfish embryos exposed to various sublethal cyanide concentrations in a continuous flow-through system from the time of fertilization to 50% hatch at 25°C.

Cyanide Conc. (mg/L HCN)	Experiment 1		Experiment 2		Experiment 3		Mean No. Hours to Hatch
	No. of Eggs	Hatching Time (Hrs)	No. of Eggs	Hatching Time (Hrs)	No. of Eggs	Hatching Time (Hrs)	
0.00	50	108	50	120	100	120	114
0.065	50	144	100	132	100	144	140
0.075	50	144	100	144	100	144	144
0.087	50	144	100	144	50	144	144
0,15	50	192	-	-	50	240	216

Table 3. Hatching success of flagfish embryos exposed to various sublethal cyanide concentrations in a continuous flow-through system from the time of fertilization to hatching at 25°C.

Cyanide Conc. mg/L HCN)	Experiment 1		Experiment 2		Experiment 3		Mean
	No. of Eggs	Hatching Success %	No. of Eggs	Hatching Success %	No. of Eggs	Hatching Success %	Hatching Success %
0.00	50	88	50	90	100	89	89
0.065	50	86	100	84	100	87	85.6
0.075	50	70	100	65	100	68	67.6
0.087	50	60	100	56	50	52	56
0.15	50	2	-	-	50	4	3

and 0.087 mg/L HCN. At 0.075 mg/L HCN, the mean hatching success was 67.6% which represents a 24% reduction compared to the controls which had a mean hatching success of 89%. At 0.087 mg/L HCN, only 56% of the embryos hatched which indicates a 48% reduction when compared to the mean hatching success of the controls. Student "t"-test indicates that the hatching success of the cyanide-exposed embryos was significantly different from that of the controls ($p \leq 0.05$). Hatching success was poor at 0.15 mg/L HCN where only 3% of the total embryos initially incubated hatched.

Effects of Cyanide on the Survival of Flagfish Larvae with Prior Exposure to Cyanide from Fertilization to Hatching

Percent survival was defined as the percent of larvae which were capable of surviving through the yolk resorption stage and reaching the time of initial feeding. The post-hatched larvae usually required 5±1 days to complete the yolk resorption process. The swim-up larvae began feeding actively on the seventh day following hatching (Cheng and Ruby, unpublished data). The survival studies of the present investigation were terminated on the tenth day following the hatching of the embryos.

The percent survival of embryos decreased with increasing cyanide concentrations. The mean percent survival of flagfish fry subjected to sublethal cyanide toxification is given in Table 4. The lowest concentration of cyanide that affected fry survival was 0.075 mg/L HCN which gave a mean survival of 91.72% which was 6% lower than the controls (96.63%). A more marked influence of cyanide occurred at 0.087 mg/L HCN in which only 83.92% of the embryos survived. Survival

Table 4. Flagfish fry survival in cyanide in a continuously renewed water system at 25 °C. Calculations of mean fry survival was based upon the number of fry hatched.

Cyanide Conc. (mg/L HCN)	Mean Survival of fry %	S.D.
0.00	97.8	±1.2
0.065	96.96	±2.1
0.075	91.72	±1.5
0.087	83.92	±2.4
0.15	0	–

Table 5. Yolk conversion efficiency of flagfish fry following removal of embryos upon hatching from sublethal cyanide exposure.

Cyanide Conc. (mg/L HCN)	Rate of (a) growth in length (mm.)	(b) yolk resorption (mm^3)	Growth per unit yolk resorbed a/b	Efficiency of yolk resorption a/b x 100
0.00	0.7	3.0835	0.227	22.7%
0.065	0.217	1.457	0.1489	14.89%
0.075	0.53	5.305	0.0998	9.98%
0.087	0.316	7.174	0.044	4.4%

in this group was reduced by 14% as compared to the controls. Statistical analysis showed a significant difference in percent survival between the cyanide treated and the control embryos ($p \leq 0.05$). Severe damage was observed at concentration of 0.15 mg/L in which none of the hatched embryos a total of 3%, survived more than 48 hours beyond the post hatching period.

Effects of Cyanide on the Efficiency of Yolk Utilization in Flagfish Fry

The effects of cyanide on the efficiency of yolk utilization was expressed by the ratio of growth rate of larvae to yolk resorption rate which gives a measure of the amount of growth per unit of yolk resorbed.

The results indicate a reciprocal relationship, with yolk conversion efficiency declining as cyanide concentration increases. Embryos exposed to 0.087 mg/L HCN had very poor yolk resorption efficiency (Table 5.). A value of 4.4% compared to 22.7% in the control fish. At 0.075 mg/L HCN, embryos were able to resorb yolk more efficiently; a value of 9.98% was obtained in this treatment. As the cyanide concentration decreased to 0.065 mg/L HCN, the efficiency of yolk conversion was accordingly increased; a rate of 14.89% was recorded in this group. Yolk conversion efficiency in embryos of all cyanide concentrations tested was significantly different from that of the controls ($p \leq 0.05$). This response of yolk conversion efficiency of flagfish embryos to cyanide strongly implied a dose-dependent response.

Growth of Flagfish Fry Following Prior Exposure to Hydrogen Cyanide During Embryonic Stage

Differential growth rate was observed between the previously cyanide treated and control fry. An obvious increase in size of the treated groups (0.065, 0.075, and 0.087 mg/L HCN) was noticeable as the

fry grew. Determination of different growth rates of flagfish larvae during the post hatching period was based upon three sets of measurements of both length and weight of larvae recorded at different ages. The first measurement was recorded on day 0 shortly following the hatching of the fry, while the second and the third measurements were carried out on the 14th and 28th day during the post hatching period respectively.

Lengths recorded on day 0 post hatching showed that fry which had been exposed to 0.065, 0.075, and 0.087 mg/L HCN measured 3.105 mm., 3.145 mm., and 3.055 mm. respectively, whereas fry from the corresponding control measured 3.120 mm. (Table 6.). Statistical analysis showed that the mean lengths of treated fry were not significantly different from that of the controls at day 0 ($p \leq 0.05$). At the same cyanide concentrations (0.065, 0.075, and 0.087 mg/L HCN), the mean length of the 14 day old fry was 0.98 cm., 1.00 cm., and 1.03 cm. for the treated groups, compared to a mean length of 0.90 cm. in the controls (Table 7.). The average length from the three cyanide treated showed a gain of 0.10 cm. in length per fish relative to the controls. By 28 days post hatching period, mean lengths of larvae from the treated groups (0.065,0.075, and 0.087 mg/L HCN) were 1.91 cm., 1.95 cm., and 2.09 cm. respectively. This represents a mean gain of 0.30 cm. in length per fish compared to the average control fish which measured 1.69 cm. in length (Table 7.). Statistical analysis showed that the mean lengths of the treated groups of both the 14 and 28 day old fry were significantly different than the corresponding control fish ($p \leq 0.05$).

The mean length of fry from the three measurements recorded during day 0, day 14, and day 28 post hatching were utilized to express the absolute growth in length, which is defined as the average total

length at each age; fry at hatching, 14-day old fry, and 28-day old fry. The relative growth of fry was computed by dividing the difference in length of the mean of two different age groups by time (in days). Two intervals of relative growth in length of fry were obtained; the first interval was measured starting from fry at zero age to fry 14 days old. and the second interval started from fry at 14 days old to 28 days old. Computed mean relative growth rate of larvae of the two defined intervals are presented in Table 8. Results of the computations indicate the values of relative growth rate of larvae from day 0 to day 14 (1st interval) were 4.7, 4.8, and 5.1% respectively of larvae previously treated with 0.065, 0.075, and 0.087 mg/L HCN which significantly differed ($p \leq 0.05$) from that of the control which was 4.2%. Similarly, the relative growth rates ofthe treated larvae measured in the second interval were 6.6, 6.7, and 7.5% respectively of larvae from 0.065, 0.075, and 0.087 mg/L HCN compared to 5.6% of that from the control group. Statistical analysis showed that the values obtained from the treated groups were significantly different than that of the control ($p \leq 0.05$). The increase in relative growth rate among the treated groups (0.065, 0.075, and 0.087 mg/L HCN) appeared to be constant between the two intervals (Fig. 10).

Unlike the measurement of length, it was technically impossible to obtain precise measurements of weights of newly hatched flagfish larvae (day 0). Only two sets of measurements were recorded on the weights of larvae at two different ages: the 14-day old and 28-day old fry. Values of measurements are presented in Table 7. Weights recorded on the 14th day post hatching showed that fry previously exposed to cyanide concentrations of 0.065, 0.075, and 0.087 mg/L during their embryonic stage

Table 6. Length of flagfish fry at hatching (mm.) following incubation of the embryos in sublethal cyanide concentrations from time of fertilization until hatching in a continuously renewed water system at 25 °C.

Cyanide Conc. (mg/L HCN) sample	0.00	0.065	0.075	0.087
1	3.50	3.50	3.00	3.75
2	3.20	2.75	3.50	2.95
3	2.65	2.55	3.25	2.75
4	3.20	3.20	3.50	2.75
5	3.00	3.40	2.75	3.60
6	3.00	3.25	3.00	2.55
7	3.55	3.25	3.00	2.25
8	2.90	2.95	3.25	3.20
9	3.20	3.00	3.00	3.25
10	3.00	3.20	3.20	3.75
Σ	31.20	31.05	31.45	30.55
$\bar{X}$	3.120	3.105	3.145	3.055

Table 7. Length and weight of flagfish fry following removal of embryos upon hatching from sublethal cyanide exposure from time of fertilization until hatching in a continuously renewed water at 25°C. The length was measured in cm. and the weight in grams.

Cyanide Conc.	0.00				0.065				0.075				0.087			
(mg/L HCN)	14-day		28-day		14-day		28-day		14-day		28-day		14-day		28-day	
Sample	Lt.	Wt.	Lt.	Wt.	Lt.	Wt.	Lt.	Wt.	Lt.	Wt.	Lt.	Wt.	Lt.	Wt.	Lt.	Wt.
1	0.6	1.2	1.7	2.2	1.1	1.3	1.8	2.5	1.1	1.2	2.0	2.6	1.3	1.3	2.3	2.4
2	1.2	1.1	1.5	1.8	1.1	0.85	2.0	2.3	1.2	1.4	1.9	2.1	1.25	1.25	2.2	2.2
3	1.0	1.0	1.7	2.1	0.9	1.2	2.1	2.6	0.9	1.4	1.7	1.8	1.35	1.2	1.9	2.3
4	1.2	0.9	1.9	2.3	0.8	1.0	1.9	2.6	0.85	0.95	2.1	2.3	1.0	1.35	2.4	1.9
5	0.8	0.95	1.6	2.0	0.9	0.85	1.8	2.4	0.95	0.9	2.0	2.5	1.0	1.4	2.0	2.5
6	0.8	0.8	1.6	1.85	1.1	1.3	1.7	1.9	0.9	1.1	1.9	2.3	0.85	0.9	1.8	2.6
7	1.0	0.9	1.3	1.7	1.1	1.25	2.1	0.7	1.3	2.0	2.5	0.8	1.4	2.2	2.1	1.9
8	0.85	1.1	2.1	2.2	0.8	1.35	2.0	2.3	1.2	1.3	1.3	2.2	1.9	1.0	1.3	2.6
9	0.75	1.1	1.8	2.3	0.9	1.3	2.1	2.1	1.1	1.1	1.8	2.2	1.0	1.25	2.1	2.1
10	0.8	1.2	1.7	1.9	1.1	1.3	1.6	1.95	1.1	1.2	1.9	2.8	0.75	1.35	1.8	2.3
$\bar{X}$	0.9	1.02	1.69	2.02	0.98	1.17	1.91	2.28	1.0	1.18	1.95	2.32	1.03	1.27	2.09	2.26
S.D.	0.18	0.13	0.21	0.21	0.12	0.18	0.15	0.24	0.16	0.16	0.14	0.29	0.33	0.13	0.18	0.24
Length-weight Relationship	1.4		0.42		1.243		0.326		1.185		0.313		1.16		0.255	

Table 8. Absolute and relative growth of flagfish following removal of embryos from sublethal cyanide upon hatching.

Cyanide Conc. (mg/L HCN)	Mean total length of fry			Mean relstive growth rate per day %	
	at hatching (cm.)	14-day old post-hatching (cm.)	28-day old post-hatching (cm.)	0-14 days post-hatching	14-28 days post-hatching
0.00	0.312	0.90	1.69	4.2	5.6
0.065	0.3105	0.98	1.91	4.7	6.6
0.075	0.3145	1.00	1.95	4.8	6.7
0.087	0.3055	1.03	2.09	5.1	7.5

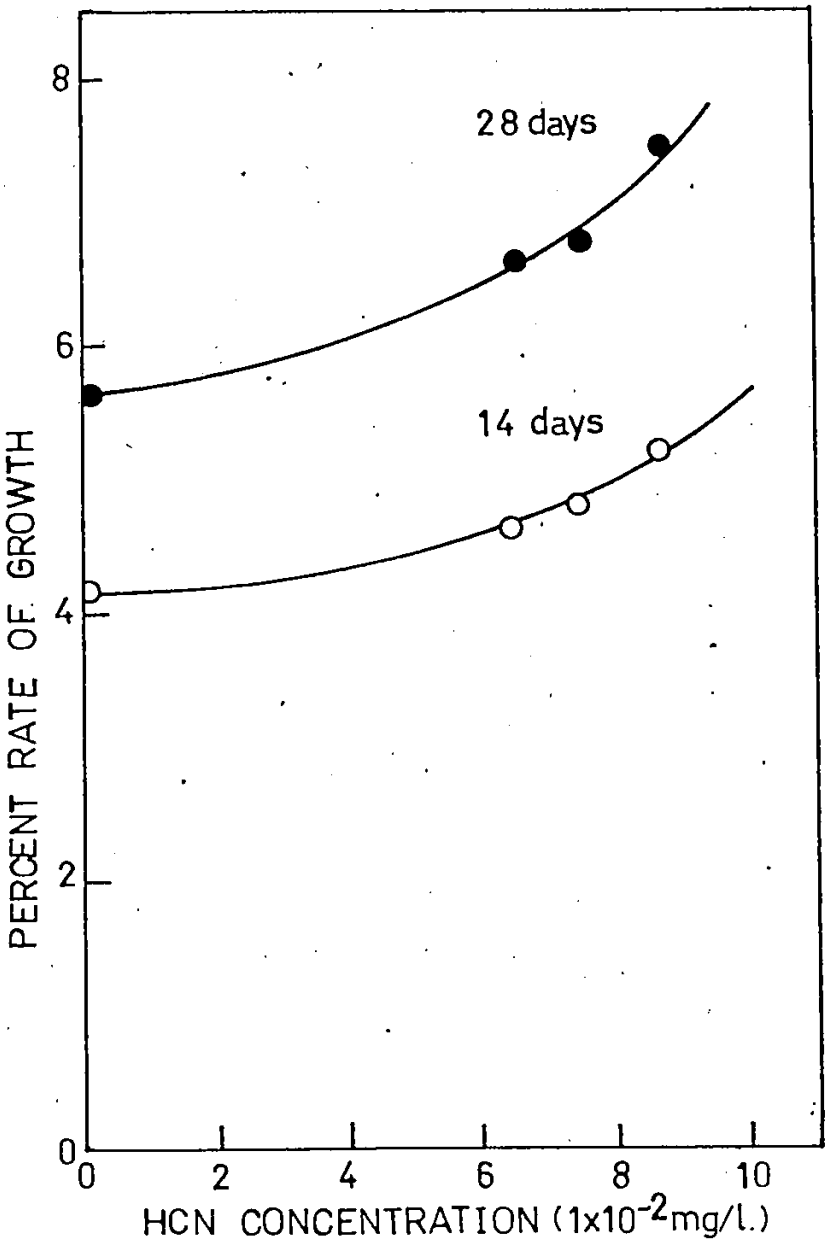


FIGURE 10. PERCENT GROWTH RATE (ABSOLUTE GROWTH) OF FLAGFISH FRY AT 14 AND 28 DAYS POSTHATCHING FOLLOWING EXPOSURE TO CYANIDE DURING EMBRYONIC STAGE AT 25°C.

had an average weight of 1.17, 1.18, and 1.27 grams per fish respectively, while the average weight for the control fish was 1.02 grams. Mean difference between the treated and the control groups indicate an average gain of 0.2 gram per fish by the treated groups during the initial 14 days post hatching, assuming no differences in control and treated embryos at day 0. Records of weights obtained on the 28th day post-hatch indicate that a sample of ten fry from 0.065, 0.075, and 0.087 mg/L HCN weighed 2.28, 2.32, and 2.26 grams. This represents a mean gain of 0.26 gram in weight compared to an average control fish which weighed 2.03 grams. Statistical analysis showed that the mean weights of the cyanide treated groups in both the 14 and 28-day old fry were higher than the corresponding controls ($p \leq 0.05$). The weight of the treated embryos on day 14 and 28 did not vary significantly among the corresponding samples.

The weights of fry at day 14 and 28 were used along with the corresponding length in the calculation of a weight-length relationship of both the treated and the control groups. The results of the calculation are presented in Table 7. Measurement made on the 14th day post hatching showed that the controls had a weight-length relationship of 1.4, whereas those of the treated groups (0.065, 0.075, and 0.087 mg/L HCN) were 1.243, 1.185, and 1.16 respectively. Similarly, measurements recorded on the 28th day of the post hatching period showed that the controls had a weight-length relationship of 0.421 while the respective values for the experimental fry from 0.065, 0.075, and 0.087 mg/L HCN treatments were 0.326, 0.313, and 0.255. The mean values of weight-length relationship of the treated groups measured on both 14 and 28 days post hatching were lower than those of the corresponding controls. Statistical

analysis revealed that the weight-length relationship of the control group were significantly higher than the cyanide treated groups at day 14 and 28 ($p \geq 0.05$).

Incidence of abnormalities

Microscopic examination of histological sections prepared from embryos previously treated with cyanide from fertilization to hatching revealed the presence of similar pituitary gland when compared to the controls. Measurements of the pituitary gland sections indicated mean maximum width of the pituitary gland of fry exposed to 0.065, 0.075, and 0.087 mg/L HCN was 0.081 mm., 0.076 mm., and 0.09 mm. respectively, while the control group mean was recorded as 0.147 mm. and 0.149 mm. in width and length respectively (Table 9). Results from statistical analysis indicates that the maximum width and length of the pituitary galnds in treated fry were significantly different from the controls.

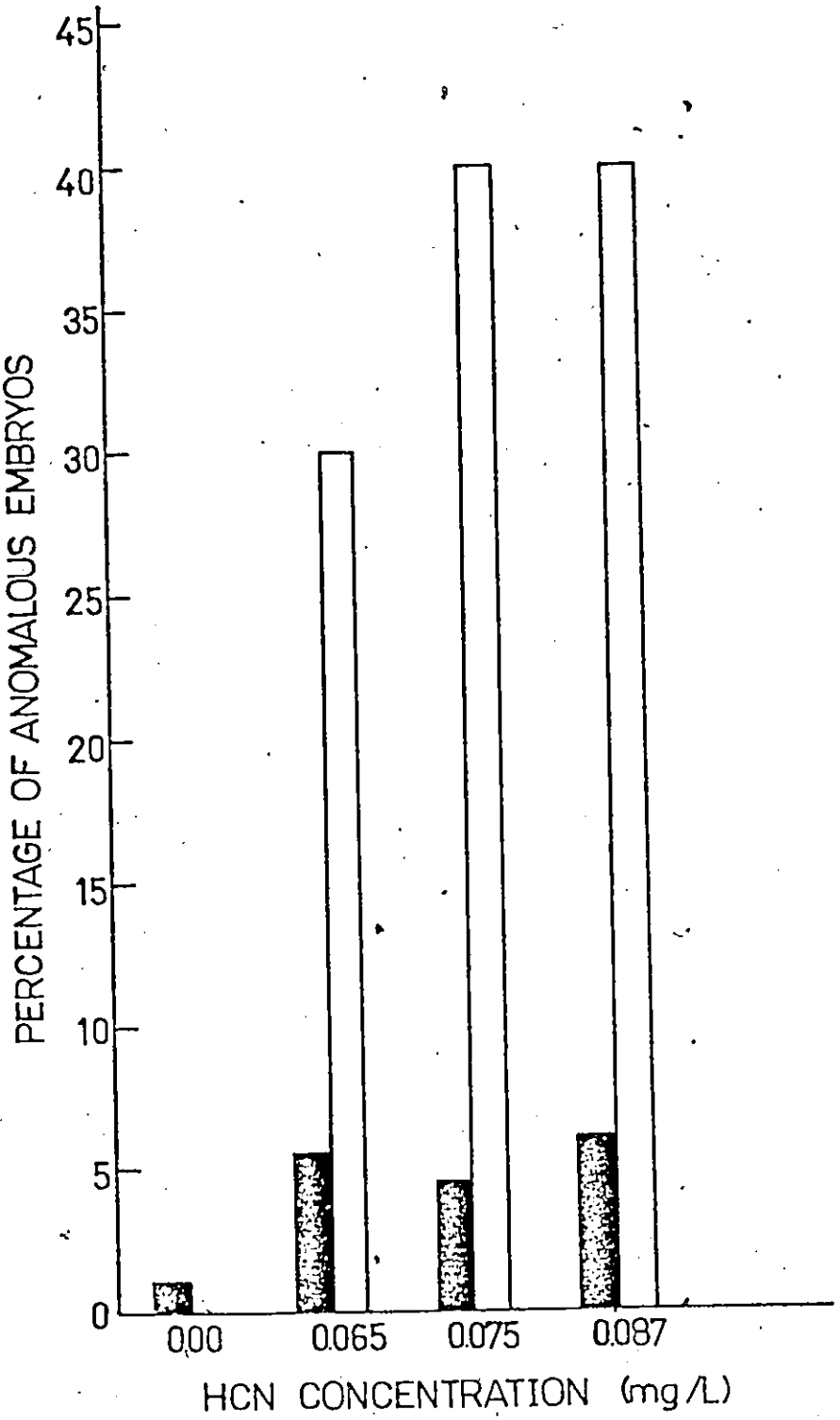
Other cyanide-induced abnormalities included eye defects. Microphthalmia and monophthalmia, the alteration of optical layers resulting in a reduction in the size of eye, i.e. microphthalmia, or a complete disintegration of an eye, i.e. monophthalmia, were common among the cyanide treated embryos. Results of this eye defect are presented in Figure 12. In 0.065 mg/L HCN, the combined incidence of micro-, and monophthalmia was 30%. In fry exposed to 0.075 and 0 .087 mg/L HCN, the eye defect incidence was not markedly different from the fry previously treated in 0.065 mg/L HCN ($p \geq 0.05$).

A 40% of the combined incidence of both the micro-, and monophthalmia

Table 9. Maximum width and length of the pituitary gland (mm.) in flagfish embryos following exposure to cyanide from fertilization through hatching.

Cyanide Conc. (mg/L HCN)	0.00		0.065		0.075		0.087	
Sample	width	length	width	length	width	length	width	length
1	0.13	0.15	0.07	0.09	0.08	0.11	0.11	0.10
2	0.15	0.20	0.095	0.10	0.07	0.07	0.09	0.07
3	0.11	0.20	0.09	0.08	0.06	0.10	0.12	0.09
4	0.12	0.13	0.05	0.09	0.08	0.09	0.08	0.07
5	0.13	0.15	0.06	0.11	0.07	0.11	0.11	0.11
6	0.15	0.17	0.11	0.11	0.06	0.06	0.07	0.10
7	0.13	0.14	0.08	0.08	0.07	0.07	0.07	0.06
8	0.20	0.12	0.06	0.06	0.12	0.09	0.08	0.12
9	0.12	0.13	0.09	0.08	-	0.10	0.10	0.08
10	0.13	0.10	0.10	0.07	-	0.11	0.13	0.10
$\overline{X}$	0.147	0.149	0.081	0.087	0.076	0.082	0.09	0.09
S.D.±	0.246 x 10^{-2}	0.3113 x 10^{-2}	0.2864 x 10^{-2}	0.155 x 10^{-2}	0.1798 x 10^{-2}	0.0184 x 10^{-2}	0.433 x 10^{-2}	0.184 x 10^{-2}

FIGURE 12. HISTOGRAM SHOWING THE EFFECTS OF THREE CONCENTRATIONS OF CYANIDE, 0.065, 0.075, AND 0.087 MG/L, ON THE INCIDENCE OF ANOMALOUS DEVELOPMENT OF FLAGFISH LARVAE INITIALLY SUBJECTED DURING THE EMBRYONIC STAGE FROM FERTILIZATION THROUGH HATCHING. OPAQUE AREAS DEPICT THE PERCENTAGE OF EMBRYOS WITH BODY FLEXURES. UNSHADED AREAS INDICATE THE PERCENTAGE OF EMBRYOS WITH EYE DEFECTS.


45
40
35
30
25
20
15
10
5
0
PERCENTAGE OF ANOMALOUS EMBRYOS
0.00
0.065
0.075
0.087
HCN CONCENTRATION (mg/L)

was recorded in both of these higher concentrations.

The larvae suffering from micro-, and monophthalmia were not easily detected at ahtching. Symptoms usually became apparent when larvae reached 14 days or more post hatching. Fish with this defect were observed to swim with a circular motion with one side up. Mortality during the first week of post hatching time considerably reduced the true incidence of eye defect in the treated embryos.

Body flexure was also a common morphological defect in the cyanide exposed embryos. Flexure usually occurred in the caudal region of the larvae (plate 4). For the purpose of quantifying the incidence of this defect a randomly selected subsample of twenty larvae was taken from both the controls and the cyanide treated groups. The incidence of body flexure is expressed in a histogram in Figure 12. At 0.065 mg/L HCN, fry with caudal flexure represented 55% of the total sample. The incidence of body flexure in fry exposed to 0.075 and 0.087 mg/L HCN were 45% and 60% respectively. Controls demonstrated fewer than 1% (0.5%) incidence of this defect. Affected were normally inactive, and generally remained at the bottom of the test tanks. These fish did not survive beyond the first week during the post hatching period.

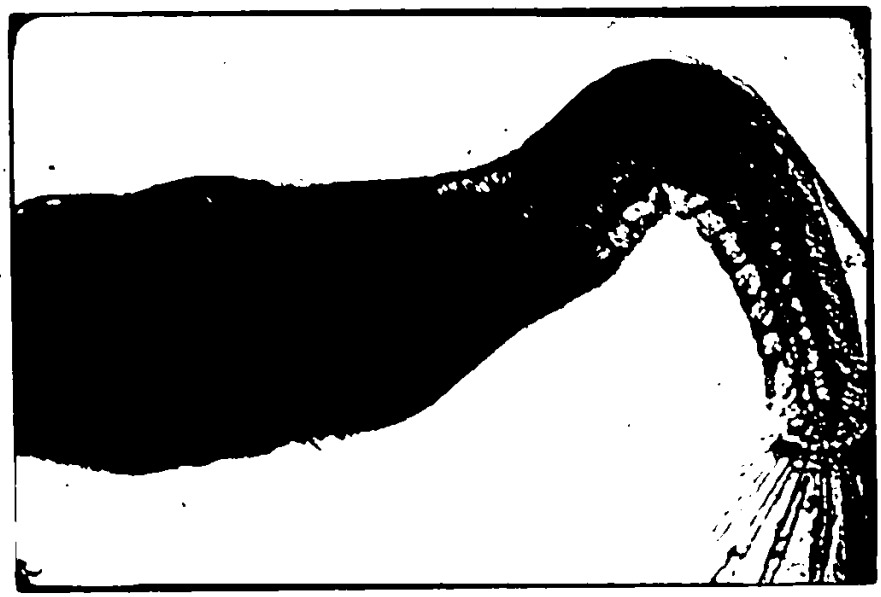


Plate 4. An illustration of a defected flagfish larva suffering from permanent body flexure following cyanide exposure at 0.15 mg/L HCN from fertilization through to hatching.

Studies of the Effects of Exposure of Flagfish During the Embryonic and Juvenile Stages to Sublethal Hydrogen Cyanide Concentrations of 0.065, 0.075, and 0.087 mg/L HCN

Treatment 1

Fish which received a single exposure to 0.065, 0.075, and 0.087 mg/L HCN during the embryonic stage (group A) (Chart 2.) displayed a 14±2 day delay in sexual maturity when compared to the controls. The first appearance of eggs on the spawning mat normally did not occur earlier than the 70th day following hatching whereas all control females commenced spawning no later than 56 days following hatching. Spawning occurred as usual, however, in all the exposed females. The estrous cycle was shortened from the normal 12-day duration in the controls to 8 days in all cyanide treatments. For the purpose of this study, estrous cycle is defined as the duration of egg laying starting from the first appearance of more than ten eggs through the first appearance of less than ten eggs on the mats. The fecundity of females and the computed total number of eggs yielded per female per estrous cycle at different concentrations are summarized in Table 10.

The total number of eggs laid in cyanide exposed fish was reduced. Table 10 shows that the total mean egg yield by the controls in experiments 1 and 2 was 1126, whereas treated fish produced 838, 753.5, and 727.5 eggs respectively in cyanide concentrations of 0.065, 0.075, and 0.087 mg/L HCN. This represents a mean total reduction of 353 eggs compared to the controls. The maximum number of eggs laid per female at the peak of the estrous cycle was also significantly reduced in the cyanide exposed fish. Maximum number of eggs laid were 243 and 241 for the control groups whereas in all the treated groups, the maximum

number of eggs laid were respectively 124 and 172 in the fish exposed to 0.065 mg/L HCN, 197 and 201 in fish exposed to 0.075 mg/L HCN, and 212 and 195 in fish treated with 0.087 mg/L HCN. The maximum number of eggs laid for all the cyanide treated groups did not exceed 2oo at the peak of the estrous cycle. This represents a mean reduction of 50±10 eggs compared to the controls.

Females which received an exposure to hydrogen cyanide during the embryonic stage and a second exposure during the juvenile stage (group B) (Chart 2) for five days demonstrated a similar initial response as the previous groups,such as retardation in fecundity. Sexual maturity was delayed 14±2 days. The average length of estrous cycle was identical to group A females. The mean total number of eggs produced in this group was 639.5, 770.5, and 603 at 0.065, 0.075, and 0.087 mg/L HCN respectively compared to 1142 in the control group. The F1 fry hatching success in this group (B) was relatively poor compared to both groups A and C. Studies of the Effects of the Exposure of Flagfish During the Embryonic and Adult Stages to Sublethal Hydrogen Cyanide Concentrations of 0.065, 0.075, and 0.087 mg/L HCN.

Treatment 2

Results of fecundity in females which were exposed to hydrogen cyanide during the embryonic stage(group A) (Chart 3) have been discussed in Treatment 1. As outlined previously, sexual maturity in the treated groups was delayed by 14±2 days as compared to the controls. The first appearance of eggs on the spawning mats normally did not occur earlier than 70 days following hatching, whereas all the controls commenced spawning no later than 56 days following hatching. The estrous cycle

was shortened from the normal 12-day duration in the controls to 8 days in this treatment.

The total mean egg yield in the controls and the treated groups (0.065, 0.075, and 0.087 mg/L HCN) and the maximum number of eggs produced per female at the peak of estrous cycle appears in Table 11.

Females which received exposure to hydrogen cyanide during embryonic stage and a second five day exposure at sexual maturity (group C) (Chart 3) did not differ significantly from those which were exposed to hydrogen cyanide only during embryonic stage ($p \geq 0.05$). Similarly, sexual maturity was delayed 14±2 days. The average length of the estrous cycle was shortened to 8 days as compared to the 12-day duration in the controls. The mean total number of eggs produced in this group was lower than the controls. Table 12 shows that the control fish produced an average of 1379 eggs per female per estrous cycle whereas fish following treatment with 0.065, 0.075, and 0.087 mg/L HCN during embryonic and adult stages yielded mean values of 901, 1176, and 1064.5 eggs respectively. In comparison, the control fish produced 300±30 more eggs per estrous cycle per female than the treated fish. The maximum number of eggs yielded per female at the peak of the estrous cycle in the two control experiments was 271 and 288 respectively, while in the treated groups (0.065, 0.075, and 0.087 mg/L HCN), values ranged from 173 to 257. The overall egg production was not significantly different from that of the controls ($P \geq 0.05$) (Table 12).

The mean hatching time of embryos, from parents previously exposed to cyanide during various stages of development (groups A, B, and C) was identical to that observed in the controls (Table 13). Values obtained for hatching success in group B, however, were significantly

lower than those of both groups A and C ($p \leq 0.05$) (Tables 14 and 15).

Table 10. Effects of cyanide on the fecundity of individual female flagfish which were exposed to cyanide during embryonic stage from time of fertilization until hatching (group A). Fecundity was expressed as the number of eggs laid/female/day.

Cyanide Conc. (mg/L HCN)	0.00		0.065		0.075		0.087	
	Expt.1	Expt.2	Expt.1	Expt.2	Expt.1	Expt.2	Expt.1	Expt.2
Day 1	0	0	0	0	0	0	0	0
2	7	43	0	8	0	14	0	0
3	11	118	17	74	89	66	7	2
4	15	179	25	105	132	111	46	0
5	21	241*	4	172*	150	114	141	96
6	244*	228	42	161	197*	201*	212*	128
7	104	102	124*	102	107	45	90	34
8	74	82	97	88	148	79	91	195*
9	75	54	33	62	24	13	52	97
10	169	117	6	49	0	0	146	18
11	119	95	0	17	0	17	68	15
12	77	40	3	0	5	0	7	2
13	19	0	0	0	0	0	0	5
14	0	3	0	0			5	7
15	8	0					0	0
Σ	934	1317	384	838	847	660	860	593
$\bar{X}$	1126		838		753.5		727.5	

* Values represent maximum eggs laid per female at the peak of estrous cycle.

Table 11. Effects of cyanide on the fecundity of individual female flagfish which were exposed to cyanide during embryonic and juvenile stage (group B). Fecundity was expressed as the number of eggs laid/female/day

Cyanide Conc. (mg/L HCN)	0.00		0.065		0.075		0.087	
	Expt.1	Expt.2	Expt.1	Expt,2	Expt.1	Expt.2	Expt.1	Expt.2
Day 1	17	0	0	0	0	5	0	0
2	78	63	0	27	13	9	4	17
3	64	157	15	128	5	3	0	58
4	115	284*	34	117	28	41	8	25
5	186	102	89	214*	64	17	10	77
6	235*	119	108	48	57	98	27	215*
7	195	85	172*	76	179	154	119*	112
8	43	147	114	18	210*	181*	36	53
9	71	57	21	12	91.	116	70	81
10	22	8	0	22	34	49	27	32
11	50	27	14	6	72	62	18	9
12	44	14	9	17	25	116	0	0
13	18	0	0	19	11	108	6	0
14	14	6	0	0	3	8	0	0
15	9	3				14	12	
16	0	0				0	0	
17	25	17						
18	0	9						
Σ	1186	1098	576	703	792	746	437	779
$\bar{X}$	1142		639.5		770.5		603	

*Values represent maximum eggs laid per female at the peak of estrous cycle.

Table 12. Effects of cyanide on the fecundity of individual female Flagfish which were exposed to cyanide during embryonic and adult stage (Group C). Fecundity was expressed as the number of eggs laid/female/day

Cyanide Conc.	0.00		0.065		0.075		0.087	
(mg/L HCN)	Expt.1	Expt.2	Expt.1	Expt.2	Expt.1	Expt.2	Expt.1	Expt.2
Day 1	0	0	12	0	0	0	0	0
2	25	0	40	0	0	23	0	35
3	81	101	56	5	17	147	51	5
4	107	158	113	29	118	172	8	129
5	192	213	109	143	45	197	17	154
6	271*	228*	185*	204	131	208	82	143
7	176	161	76	257*	195*	216*	173*	102
8	191	134	121	121	81	116	116	215*
9	138	147	37	95	65	152	94	142
10	56	79	19	108	153	76	151	119
11	11	41	0	56	96	2	0	85
12	38	118	0	16	20	25	4	13
13	8	84	8	10	53	19	75	27
14	0	13	0	5	11	0	61	3
15	3	0	2	17	15	3	9	4
16	0	9	0	0	0	11	0	11
17	0	7	0	0	0	0	0	0
Σ	1294	1464	768	1034	1000	1352	841	1287
$\bar{X}$	1379		901		1176		1065.5	

* Values represent maximum eggs laid per female at the peak of estrous cycle.

Table 13. Mean hatching time of flagfish embryos following exposure of parents to hydrogen cyanide in continuously renewed water system from time of fertilization to 50% hatch at 25 ℃.

Cyanide Conc. (mg/L HCN)	Treatment 1 hatching time (hours)	Treatment 2 hatching time (hours)
0.00	114	114
0.065	114	114
0.075	114	114
0.087	114	114

Table 14. Mean hatching success and survival of flagfish embryos following exposure of parents to hydrogen cyanide during embryonic and juvenile stages in continuously renewed water at 25 ℃.

Cyanide Conc. (mg/L HCN)	Embryonic exposure (Group A)		Embryonic and juvenile exposure (Group B)	
	Hatching success %	Survival %	Hatching success %	Survival %
0.00	90.5	96.8	89.0	97.2
0.065	89.0	97.0	76.0	96.5
0.075	88.0	96.7	75.5	97.1
0.087	89.0	98.2	77.0	97.5

Table 15. Mean hatching success and survival of flagfish embryos following exposure of parents to hydrogen cyanide during embryonic and adult stages in continuously renewed water at 25°C.

Cyanide Conc. (mg/L HCN)	Embryonic exposure (Group A)		Embryonic and adult exposure (Group C)	
	Hatching success %	Survival %	Hatching success %	Survival %
0.00	90.5	96.8	90.0	97.7
0.065	89.0	97.0	91.0	96.0
0.075	88.0	96.7	89.0	96.9
0.087	89.0	98.2	88.0	96.7

Acute 96-hour Bioassays of Flagfish Fry of Different Age Groups to Hydrogen Cyanide

The results of the 96-hour acute bioassays are presented in Figure 13. Data were analysed and computed after Litchfield and Wilcoxon (1949). Each point represents the median mortality rate of thirty fish (in triplicates). The 24-hour old fry proved to be a most critical at all the concentrations of cyanide tested (0.03 to 1.0 mg/L HCN). They survived the shortest time and had the highest mortality rate in the 4-day bioassay. The cyanide concentration which caused 50% mortality of this group in ninety-six hours was 0.42 mg/L HCN. Resistance increased steadily with age. The 48-hour old fry were more resistant than the 24-hour fry. The cyanide concentration which caused 50% mortality in ninety-six hours was 0.44 mg/L HCN. While the 72-hour old fry and 96-hour old fry were highly resistant in comparison to the 24- and 48-hour old fry. They reached 50% mortality at cyanide concentrations of 0.65 and 0.73 mg/L HCN respectively.

The overall results indicate that the 24- and 48-hour old fry are highly sensitive to cyanide, while the 72- and 96-hour old fry were highly resistant to cyanide. At cyanide concentrations of 0.37, 0.49, 0.57, and 0.65 mg/L HCN, approximately 90% of the larvae used in each of these groups survived after the termination of the bioassays.

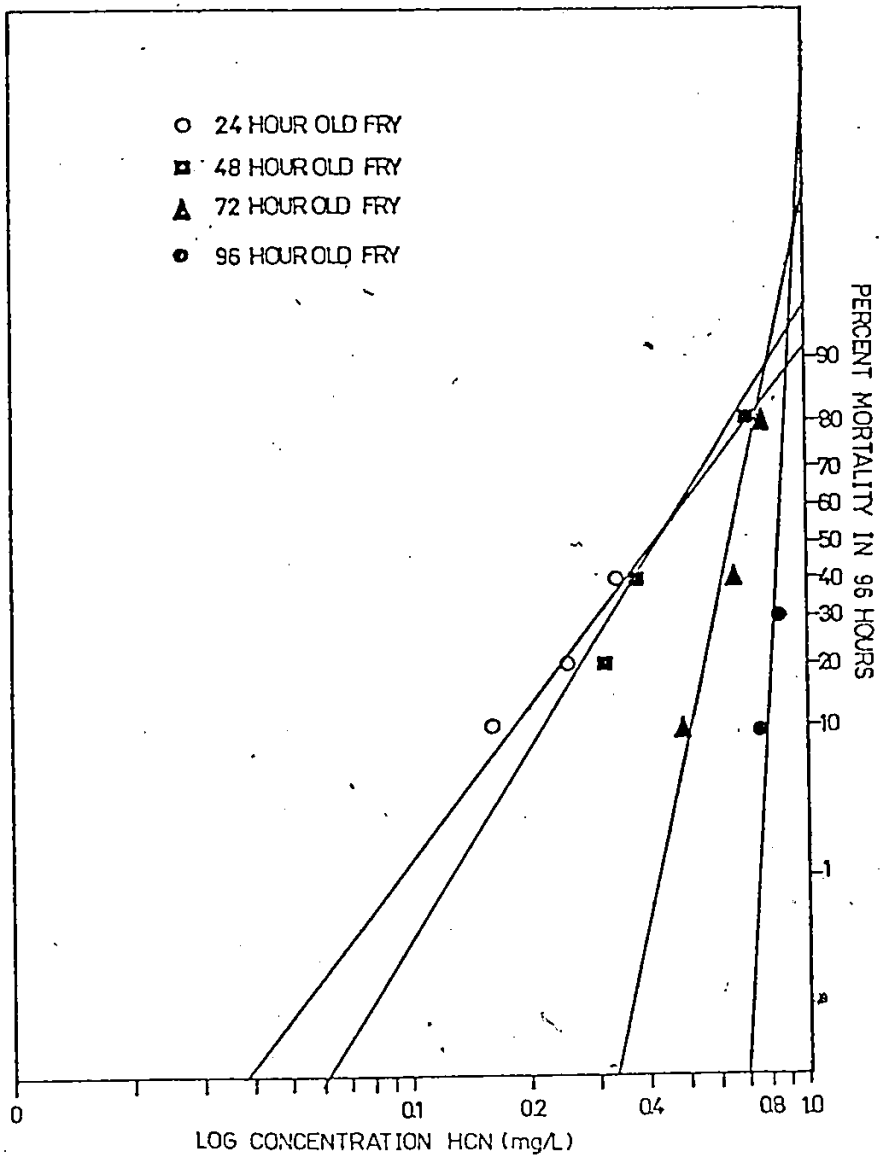


FIGURE 13. RESULTS OF ACUTE BIOASSAY TO FLAGFISH LARVAE OF FOUR AGE GROUPS; 24, 48, 72, AND 96 HOUR-OLD, ASSAYED IN A CONTINUOUS FLOW-THROUGH SYSTEM AT 25° C.

DISCUSSION

Effects of Cyanide on Hatching Time of Flagfish Embryos

There is only one previous report in the literature on the effects of chronic cyanide exposure upon hatching time in fish embryos. In that investigation, Leduc (1978) incubated Atlantic salmon embryos in cyanide concentrations ranging from 0.01 mg/L to 0.1 mg/L HCN at 2.7°C. Although some cyanide concentrations were similar to this study, nevertheless, experimental temperatures differed from the present investigation. This is the first report of the sublethal effects of cyanide on hatching time in a warm water species at 25°C.

The results of the effects of cyanide on hatching time of flagfish embryos are similar to Leduc (1978) in that they suggest a dose-dependent response. Delay in hatching time was noticed at all intermediate concentrations tested (Table 1.). Two concentrations used in the present study were similar to the Atlantic salmon experiments conducted by Leduc (1978). In flagfish embryos exposed to 0.049 and 0.087 mg/L HCN, hatching time was prolonged by 20% and 23% respectively beyond the normal incubation time of 4½ days observed in the control group. The same concentrations delayed hatching time by 2% and 4% respectively when compared with the total time required by the controls (145 days) in Atlantic salmon embryos (Leduc, 1978). A comparison of the two investigations superficially suggests that flagfish embryos are more susceptible to sublethal cyanide exposure, however, differences in both temperature and egg size in the two studies are possible modifying factors which contribute significantly.

Flagfish eggs clearly demonstrated smaller diameter relative

to that of Atlantic salmon, and measured 1.0-1.5 mm. in diameter, whereas Atlantic saloma eggs ranged from 5.0-6.0 mm. in diameter. The large surface to volume ratio present in a small flagfish egg would suggest a higher susceptibility to cyanide than that of small surface to volume ratio of the large Atlantic salmon egg. The amount of uptake, however, of toxicant and the rate of detoxification by the embryo would also represent critical determining factors which require further investigation before this question can be clearly elucidated.

Results of the hatching time suggest that flagfish embryos are particularly sensitive during gastrulation to sublethal cyanide concentrations. One of the possible explanations for the prolonged hatching time observed may be related to alterations in protein synthesis at gastrulation following exposure of the embryos to low cyanide concentrations from fertilization to hatching. Wilde and Crawford (1966) demonstrated that blastula stage of fish embryogenesis is anaerobic. It is cyanide insensitive even at concentrations as high as 520 mg/L (Wilde and Crawford, 1963). Since the formation of informational macromolecules (mRNA), which is oxygen dependent, occurs prior to fertilization, therefore, oxygen is not required for cell proliferation during the blastular stage. Protein synthesis, however, begins during gastrulation and the synthesis of mRNA is oxygen dependent. One possible explanation for the extended hatching time in fish embryos following sublethal cyanide exposure may be related to modifications in the rate of protein synthesis resulting from reduced energy levels during the gastrula stage. Alderdice, et al., (1958), Garside (1959), and Shumway, et al., (1964) have observed similar patterns in delayed hatching time following exposure of fish

embryos to reduced oxygen levels during gastrulation.

Prolonged hatching time can be mainly explained at the biochemical level through cyanide inhibition of two principle enzymes. Both the enzymes, cytochrome oxidase and succinic dehydrogenase play a major role in the entire oxidative metabolic pathways (Lehninger, 1970, p.379-p.381). Exposure of flagfish embryos to sublethal cyanide levels leads to partial paralysis of the metabolic oxidative pathways making the necessary energy unavailable. As a consequence, a slowing down of the rate of embryonic development occurs.

Effects of Cyanide on Hatching Success and Survival of Flagfish Embryos

The effects of hydrogen cyanide on the hatching success and survival of fish embryos have been frequently reported in both cold water species (Anderson, 1969, Personal communication, Leduc, 1978, and Koenst, et al., 1977) and warm water species (Lind, et al., 1977). The reports documented on hatching success of fish embryos have demonstrated that cyanide deleteriously affects the hatching success of brook trout embryos (Anderson, Personal communication), Atlantic salmon embryos (Leduc, 1978), and fathead minnow (Lind, et al., 1977) at concentrations of 0.02, 0.01, and 0.04 mg/L HCN respectively. The present results, in general, are similar in trend to the findings of the previous investigators and indicate that the hatching success of flagfish embryos in hydrogen cyanide was significantly reduced as the concentration gradient increased from 0.065 to 0.15 mg/L HCN.

Reduction in hatching success as noticed in the present study was mainly caused by mortality of the embryos prior to hatching. Mortality occurred but was less severe during the post-hatching period.

Its occurrence may be related to impairment caused by cyanide imposed during prehatching development. This postulation gains support from the findings of Anderson (1969, personal communication) that embryos of brook trout exposed to the identical cyanide concentration before or throughout the entire hatching process did not differ in mortality.

The phenomenon of partial hatching in the emerging fish embryos as induced by stress has been reported frequently in the literature. Alderdice and Forrester (1971) demonstrated that low incubation temperature caused partial hatching in Pacific herring, Clupea harengus pallasi. Similarly, some toxic chemicals such as cadmium (Rosenthal and Alderdice, 1971, and Mounib, et al.,1975) and ammonium hydroxide (Burkhalter and Kaya, 1977) also have been observed to induce partial hatching in herring, Clupea harengus harengus, and rainbow trout, Salmo gairdneri.

The present data indicate that 0.15 mg/L hydrogen cyanide produced partial hatching in flagfish embryos. The hatching process was arrested as the emerging larvae were attempting to release themselves from the egg shells, when only the head or the caudal region protruded. This abnormal hatching occurs presumably as a result of reduced embryonic movement and an alteration of hatching enzyme concentration in certain localized regions (Rosenthal and Alderdice, 1976). The cause of partial hatching may be related to the inhibitory effects of cyanide at the biochemical level. As stated previously, cyanide blocks two essential enzymes: cytochrome oxidase and succinic dehydrogenase. This action results in an energy deficit within the embryos. As a consequence, normal embryonic movement is altered. Subsequently, the circulation of perivitelline fluid, essential for distribution of oxygen and hatching enzyme

within a full term embryo is prevented. An uneven distribution of hatching enzyme resulted in partial hatching as observed in this study.

Effects of Cyanide on Efficiency of Yolk Utilization of Flagfish

The efficiency of yolk utilization has been recognized as a useful indicator to investigate stress on newly hatched fish embryos. Blaxter (1969) has reviewed the various methods available for the determination of yolk conversion efficiency. The technique developed by Ryland and Nichols (1967) was employed in this study since it has been specifically designed for the study of yolk utilization efficiency in small embryos. Leduc (1978) investigated the impact of sublethal cyanide on newly fertilized Atlantic salmon embryos continuously exposed to cyanide concentrations ranging from 0.01 to 0.1 mg/L HCN at 5.8°C. His results indicate that the yolk conversion efficiency ratio in Atlantic salmon embryos tended to be higher in the cyanide treated groups than in controls.

This is, in general, contradictory to the findings of the present study in which cyanide adversely affected yolk conversion efficiency in flagfish sac-larvae. The yolk conversion efficiency of the cyanide treated fry was reduced significantly for each increment of 0.01 mg/L HCN. The difference in results can be accounted for, in part, by the difference in experimental protocol. In the present study, embryos were exposed to hydrogen cyanide from fertilization through to hatching only, while Leduc (1978) exposed eggs and larvae to hydrogen cyanide beyond this stage and through the completion of yolk resorption. The high yolk conversion efficiency in the cyanide treated larvae observed by Leduc (1978) could be a result of low activity of the larvae under

continued cyanide exposure; a phenomenon which Leduc (1978) reported in his investigation. Blaxter (1969) states that low activity of fish larvae undergoing yolk resorption could result in more available energy for yolk conversion.

Growth Experiment

There have been four previous reports on the effects of chronic cyanide exposure upon the growth of fish larvae and juveniles. Two of these were conducted with cold water fish. Leduc (1978) studied the impact of sublethal cyanide on the growth of Atlantic salmon larvae from the time of fertilization until the embryos reached the 58-day old juvenile stage in cyanide concentrations of 0.01 to 0.1 mg/L HCN at 5.6 °C. He observed that although larvae at 0.08 and 0.1 mg/L HCN were shorter in length than the controls at hatching, yet they grew as well as the controls at 58 days. Koenst, et al., (1977) utilized juvenile brook trout to investigate the growth of this cold water species as influenced by hydrogen cyanide ranging from 0.005 to 0.07 mg/L HCN at 9 °C. They demonstrated that cyanide concentrations at 0.005, 0.01, and 0.02 mg/L HCN induced fish to grow faster than the controls. Although different temperatures and different choices of test fish were employed, the trend obtained in the present study was the same as the previous investigations (Leduc, 1978, and Koenst, et al., 1977).

Two additional experiments (Leduc, 1966, and Lind, et al., 1977) using warm water species, similarly demonstrated a faster growth rate in cyanide treated fish. Leduc (1978) performed a 36-day growth experiment with juvenile cichlid using cyanide concentrations ranging from 0.008 to 0.1 mg/L HCN at 25 °C. He noted that the growth rate of the cichlids exposed to cyanide levels of 0.06 to 0.10 mg/L HCN was markedly

reduced during the initial one third of the experiment. The reduction in growth in cyanide-treated fish was less pronounced in the second 12-day period. However, the cichlids at these cyanide levels (0.06 to 0.1 mg/L HCN) grew faster than the controls in the final 12-day period. The trend of faster growth rate induced by cyanide was confirmed by yet another experiment conducted by Lind, et al., (1977). They exposed fathead minnow larvae to cyanide concentrations of 0.005 to 0.1 mg/L HCN up to 56 days post-hatching at 25°C, and observed that cyanide encouraged growth in length of fathead minnow larvae at intermediate levels (0.06 to 0.08 mg/L HCN). These two experiments again suggested that cyanide promotes faster growth in juvenile fish. The data from the present investigation demonstrated a similar trend to all of the previous studies and suggests that cyanide causes a faster growth rate in flagfish larvae. Flagfish larvae previously treated with 0.065, 0.075, and 0.087 mg/L HCN grew markedly faster than the controls, eventhough the fry measured the same length at hatching. It is obvious from the five studies that although experimental conditions such as the time and duration of cyanide exposure, bioassay temperature, and the test fish were all different, the results of these investigations (Leduc,1966b, Lind, et al., 1977, Koenst, et al., 1977, and Leduc, 1978) showed a similar trend to that obtained in the present study. It is of significant interest that the faster growth rate observed in the juvenile flagfish in the present study occurred following cyanide exposure during the pre-hatched period only, while in all the previous experiments, exposure was continuous throughout development.

One of the basic factors which determines the growth rate of a fish during the early post-hatching period is the sequences of the

utilization of carbohydrates, proteins, and lipids. Blaxter (1969) reviewed several theories regarding the sequences of utilization of these various materials for energy production. The original concept of Needham (1933) was that materials were used up for energy in the sequence of:

Concept 1.

carbohydrate--------protein--------fat

The concept was supported by Devillers (1965). Hayes (1949), however, was of the opinion that the sequence in salmon egg was:

Concept 2.

	hatch		
primary fat synthesis	primary protein synthesis	secondary fat synthesis	secondary protein synthesis

The concept postulated by Hayes (1949) can be used to explain the differential growth rates as observed in the cyanide treated embryos of the present study. It is essential to realize that growth in length reflects protein synthesis. Hayes (1949) concept can thus be interpreted in respect to the faster increment in length of larvae initially exposed to cyanide prior to hatching. The interpretation can be related to the inhibitory effects of cyanide upon the post-blastula stage of the developing embryos. As previously discussed in an earlier section, the overall prehatching embryonic development was delayed, resulting in a delay in hatching time. It is thus, logical to suspect that both the protein and fat synthesis are altered during the prehatched stage. Consequently, at hatching, the initially cyanide treated fry could, for instance, have only reached the primary protein synthesizing phase whereas the corresponding control fish may have advanced to the secondary fat synthesizing phase (Concept 2.). With the removal of

cyanide upon hatching, the protein synthesis in the treated larvae would resume its normal pace, hence, a faster growth in the length as noted in the previously cyanide treated larvae immediately following the removal of the toxicant.

There has been evidence that the presence of cyanide inhibited protein synthesis. Leduc 1966b , based upon weight-length relationship of cyanide exposed cichlid in his growth experiment, postulated that cyanide may have inhibited protein synthesis while at the same time favouring dietary fat deposition when embryos were continuously exposed to cyanide during the pre- and post-hatching period. Although a faster growth rate was observed in both the present study and that of Leduc's (1966b) the magnitude in length of the treated fish in the two experiments differed. With continuous exposure of cyanide throughout the entire experimental period, Leduc(1966b) reported that the cyanide toxified fish were able to show a faster growth in length only in the final two third of the experiment. His results showed that there was no difference in length between the treated and the control fish by the end of the study. However, based upon the results from the fish which were removed from cyanide upon hatching, the initially cyanide treated fish out-lengthened the control fish upon the termination of this study.

Temporary inhibition of cyanide in an organism which is fully sensitive to this toxicant, may for some reason at a particular time, build up an alternative pathway for respiration. This pathway could compensate for the deficit in the basic respiration (Henry and Nyns, 1975). It follows that the temporary inhibitory effects of cyanide at the protein synthesizing phase of the developing embryos could possibly induce adaptation through the synthesis of an abnormally

higher amount of mitochondria. The presence of excessive numbers of mitochondria would, eventually, generate more ATP for protein synthesis following the removal of cyanide. This postulated mechanism could represent a possible explanation for the faster increase in length of the previously cyanide treated larvae.

There have been previous suggestions in the literature that cyanide exposure results in protein synthesis blockage in unfertilized rainbow trout eggs. Lesniak (1977) exposed pre-spawning rainbow trout to low hydrogen cyanide (0.01 and 0.02 mg/L HCN) at 10°C for twenty days. She noted that dispersion of the Balbiani body normally associated with protein synthesis in maturing oocytes was delayed. Cyanide is known to inhibit the activation of succinate dehydrogenase, an enzyme which is involved in the conversion of succinate to fumarate in the Krebs Cycle (Lehninger, 1970, p.379). Livni (1971) has demonstrated the presence of this enzyme in the Balbiani body of teleosts. Lesniak (1977) postulates that delayed Balbiani dispersion may represent blockage of succinate dehydrogenase by cyanide and would result in the prevention of utilization of excess amino acids. The faster growth rate in the post hatching larvae observed in the present study may represent an excess imput of amino acids being recycled and utilized following the removal of cyanide at hatching.

Incidence of Teratogenesis

Physical and chemical malformations in developing fish embryos have been frequently reported in the literature (Stockard, 1921, Briggs and Wilson, 1959, Battle and Laale, 1960, Oppenheimer, 1950, and Anderson and Battle, 1967). However, teratogenesis as induced by hydrogen cyanide has not been well documented. Only one report has been published

to date. Leduc (1978) incubated newly fertilized Atlantic salmon embryos in hydrogen cyanide concentrations ranging from 0.01 to 0.1 mg/L HCN in continuous renewed water at 5.6 C. He reported that incidence of abnormalities in the hatched embryos increased by 6-19% as cyanide concentrations increased from 0.01 to 0.1 mg/L HCN. Deformities occurres in the head, eyes, mouth parts, and vertebral column. By utilizing a different species of test fish and a higher incubation temperature (25°C), the findings of the present investigation were comparable to Leduc's (1978). Newly fertilized flagfish embryos, having been exposed to 0.065, 0.075, and 0.087 mg/L HCN at 25 C, developed smaller endocrine organ, micro- and monophthalmia, and permanent body flexure.

This is the first report in the literature related to the suppression of the enlargement of endocrine organs by toxicant. The findings indicated that cyanide treated embryos generally possessed smaller pituitary gland than the control fish. The size difference of the pituitary gland as noted in the cyanide treated larvae indicate that cyanide imposed during the organogenesis of flagfish embryonic development retards growth of the pituitary gland.

The pituitary gland is known to produce both gonadotrophic and melanocyte controlling growth hormones in fish including Anguilla, Cichlasoma, and cyprinodonts (Holmes and Ball, 1974, p.173). It is unlikely, however, that cell differentiation has taken place during this early stage of development, thus the size reduction of the pituitary gland observed following administration of toxicant probably stems from an overall reduction in cell numbers and is not related to a particular cell type. The implication of reduced pituitary development on reproduction following maturation of the embryos is discussed subsequently under

life studies.

Life Studies

Literature review indicates that there are only two reports on the chronic effects of hydrogen cyanide on fish through one complete generation. Lind, et al., (1977) determined the long term effects of cyanide on fathead minnow, Pimephales promelas, Refinesque, from hatching into the second generation juvenile stage following exposure in concentrations ranging from 0.005 to 0.1 mg/L HCN at 25 C. The second investigation, which was reported by Koenst, et al., (1977) adopted an approach similar to Lind, et al., (1977). Identical cyanide concentrations and exposure duration were used but brook trout, Salvelinus fontinalis, Mitchill, which is a cold water species,was utilized as the test fish. The present study differs from the previous studies conducted by Lind, et al., (1977) and Koenst, et al., (1977). In both investigations exposure to cyanide was continuous throughout the entire experimental period, while in the present study, exposure was restricted to three periods of approximately five days in duration; the first started from egg fertilization through to hatch, the second from egg fertilization to hatch followed by a second exposure during juvenile development, while the third exposure was designed to measure the effects of cyanide following a double exposure; one during egg development and a second at sexual maturity. Lind, et al., (1977) reported that the number of eggs laid per female in cyanide concentrations ranging from 0.019 to 0.105 mg/L were significantly reduced. They also showed that the hatching success of the eggs were reduced at cyanide concentrations ranging from 0.045 to 0.073 mg/L HCN. Similarly, Koenst, et al., (1977) noted a

reduction of the number of eggs spawned per female in cyanide concentration from 0.01 to 0.075 mg/L HCN. No fertile eggs were found in spawning females at 0.065 and 0.075 mg/L HCN.

It is interesting that the present study reveals similar findings with fewer eggs produced following a single short exposure during the embryonic development, a single exposure during embryonic and juvenile development, or a single exposure during embryonic and adult stages.

The reduced egg laying capacity in mature females following exposure to cyanide from fertilization to hatching is probably related to reduced pituitary gland size during embryonic development. The role of the pituitary gland in fish is well defined (Holmes and Ball, 1974, p.170-220). Reduction in number of a cell which is responsible for secreting follicle stimulating hormone (FSH) could result in a reduced egg laying capacity as demonstrated in the present study.

Sexual maturity has been reported to be delayed in brook trout when exposed continuously from the time of fertilization through to adulthood to cyanide concentrations ranging from 0.01 to 0.075 mg/L HCN (Koenst, et al., (1977). Delayed sexual maturity was also noted in the present study when flagfish embryos shortly following fertilization were exposed to 0.065, 0.075, and 0.087 mg/L HCN

reduction of the number of eggs spawned per female in cyanide concentrations from 0.01 to 0.075 mg/L HCN. No fertile eggs were found in spawning females at 0.065 and 0.075 mg/L HCN.

It is interesting that the present study reveals similar findings with fewer eggs produced following a single short exposure during embryonic development, a single exposure during embryonic and juvenile development, or a single exposure during embryonic and adult stages.

The reduced egg-laying capacity in mature females following exposure to cyanide from fertilization to hatching is probably related to reduced pituitary size during embryonic development. The role of the pituitary gland in fish is well defined (Holmes and Ball, 1974, p.170-p.220). Reduction in number of a cell which is responsible for secreting follicle stimulating hormone (FSH) could result in a reduced egg laying capacity as demonstrated in the present study. The prediction is evidenced by the findings of Ruby and Dixon (1974) who exposed yearling rainbow trout to 0.01 and 0.02 mg/L HCN at 10°C for twenty days and demonstrated that cyanide blocks mitotic division among spermatogonia. It is possible that similar blockage of mitotic division in oogonia which are undergoing mitosis during the juvenile development would result in fewer oogonia, subsequently reducing the total number of egg yielded.

Sexual maturity has been reported to be delayed in brook trout when exposed continuously from time of fertilization through to adulthood to cyanide concentrations from 0.01 to 0.075 mg/L (Koenst, et al., (1977). Delayed sexual maturity was also noted in the present study when flagfish embryos were exposed to 0.065, 0.075, and 0.087 mg/L HCN

from fertilization until hatching, followed by a second exposure either during juvenile or adult stage. It seems that delayed sexual maturity in the adult fish previously treated with cyanide as reported by Koenst, et al., (1977) and noted in the present investigation is an indication of retardation of developing oocytes.

There has been evidence that cyanide delays egg development in fish. Lesniak (1977) demonstrated that, when pre-spawned rainbow trout, Salmo gairdneri, were exposed to 0.01 and 0.02 mg/L HCN for 20 days, a retardation of ovary growth was noticed. By means of a quantitative approach based upon the mean maximum diameters of different stages of oocytes, she reported that cyanide at 0.01 and 0.02 mg/L HCN selectively prevents the maturation of oocytes. As a result, rainbow trout, having been exposed to cyanide had a significantly higher numbers of young oocytes compared to the numbers of mature oocytes. This findings by Lesniak (1977) lends its support to the results observed in the present experiment that a delay in sexual maturity in cyanide exposed fish is possibly due to a retardation of the developing oocytes affected by cyanide.

A single short term exposure to chronic cyanide during the embryonic stage produces similar results to those observed following exposure once during embryonic development and a second exposure at sexual maturity. Since the number of eggs produced is not significantly different, the implication is that a short term exposure to hydrogen cyanide for five days has little effect upon the developing eggs of a continuous breeder such as flagfish. It suggests that a single short term exposure during embryonic development is most critical. A reduct-

ion in the volume of pituitary tissue observed in this study following cyanide exposure from egg fertilization through to hatching suggests that pituitary damage during this period is probably responsible for the reduced levels of egg production at sexual maturity.

Lower hatchability and fry survival in the F_1 generation in cyanide concentration of 0.05 mg/L were observed by Koenst, et al., (1977) while Lind, et al., (1977) reported that cyanide concentrations at 0.04 and 0.07 mg/L HCN adversely lower the F_1 hatchability and survival of fathead minnow following a one generation exposure of the parents.

The present study reveals no differences in hatching time, hatching success, or survival of offspring whose parents had received continuous exposure to hydrogen cyanide from fertilization to hatching. This suggests that although the number of offspring from sexually mature adults were reduced, those offspring which were produced had not been affected by short term cyanide exposure of the parents. Similarly, hatching success and survival of those offspring in which parents were exposed for five days during embryonic and adult stages showed no significant differences in terms of F_1 hatchability and survival. This suggests that short term exposure to cyanide during sexual maturity has no significant effect upon offspring of the continuous breeding flagfish and correlates well with the fecundity studies under similar conditions.

Hatching success of offspring following exposure of parents to hydrogen cyanide during embryonic and juvenile stage was significantly affected. This suggests that a small number of eggs which were undergoing pre-spawning development during the juvenile stage of the parents

received damage from a brief exposure to hydrogen cyanide. These findings concur with those of Lesniak (1977) who has shown that pre-spawned eggs subjected to sublethal levels of cyanide undergo alterations in both protein synthesis and yolk deposition. In the present investigation, reduced hatching success may be an expression of damage incurred to the pre-spawned eggs during juvenile exposure.

In conclusion, the finding of this study suggests that short term exposure to hydrogen cyanide during critical stages of development can adversely retard the reproductive capability of flagfish, Jordanella floridae, at sexual maturity. The most critical periods occur when exposure takes place from fertilization to hatching and/or during juvenile development when differentiation, production, and maturation of the gonads has commenced.

The ecological implications are obvious. It would seem that most sublethal effects are biochemical in origin, that they are manifested as histological, morphological, and physiological damages, and that these damages alter the population structure through the integration of associated changes of individual survival potential. Within this framework it would seem that the ultimate tactical manoeuvre of an egg is to remain alive, whatever the cost, as long as the organism's compensatory apparatus is not overwhelmed by the imposed stress. Hence, the cost, though may not be lethal at the time of stress compensation, ultimately may result in mortality in a later stage of development. Thus the organism trades certainty of death at one stage for a lower probabilty of survival at a later stage. It is felt that further generation studies to determine the critical developmental stages in fish following toxicant exposure is recommended.

Acute Bioassay of Flagfish Fry to Hydrogen Cyanide Tested at Different Phases of its Life History

With the results obtained in the present study, indicating that cyanide adversely affects most of the parameters selected for study on the early life history of flagfish, it is of interest to understand if there is a particularly vulnerable period during the early stages of flagfish fry to acute cyanide toxicity. Literature review to date indicates that there is no record of this nature using cyanide as the test toxicant. Only one similar study has been documented. Skidmore (1964) exposed seven age groups of zebrafish (4, 6, 8, 10, 13, 40, and 100 days old) to 5, 10, 20, and 40 mg/L $ZnSO_4$ solution at 25 C. He showed that newly hatched fry were the most sensitive at all four concentrations of zinc sulphate tested. He concluded that the newly hatched fry of zebrafish, Brachydanio rerio, were most sensitive in the life span of the fish. The tolerance of zebrafish to zinc sulphate seemed to increase with age.

It is apparent from the data obtained in this study, by exposing four age groups of newly hatched flagfish (24, 48, 72, and 96 hours old) to 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 mg/L as HCN at 25 C, that newly hatched fish (24 hours old following hatching) were the most sensitive developmental stage in the life history of flagfish. Resistance of the fry to acute hydrogen cyanide increased steadily with age.

The overall results of the present study agreed well with those of Skidmore (1964) who observed in zebrafish that a progressive tolerance to toxicant was developed with the continued growth of fish.

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