Ammonia and Urea Production of Coho Salmon under Hatchery Conditions

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AMMONIA AND UREA PRODUCTION OF COHO SALMON UNDER HATCHERY CONDITIONS

by

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Department of Environment

Environmental Protection Service and Fisheries Service

Pacific Region

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ABSTRACT

Routine measurements of the ammonia and urea production of hatchery reared coho salmon have been carried out at the Big Qualicum River Experimental Hatchery. Concentrations of the major nitrogen excretory product, ammonia, follow a regular diurnal pattern. Urea nitrogen output is more variable and accounts for an average of less than 40% of the nitrogen excretory wastes. A variety of hatchery conditions effect characteristic changes in ammonia production. Definite increases in ammonia output have been associated with increasing water temperatures, and typical hatchery disturbances, (e.g. fish removals). The influence of high pond loading rates on ammonia output is also discussed. The possible effects on stamina, disease resistance and growth rates are related to un-ionized ammonia exposure. Un-ionized ammonia concentrations in the rearing pond have been calculated from the water temperature, pH and total ammonia concentrations.

TABLE OF CONTENTS

		PAGE
ABSTRACT		i
TABLE OF CONTENTS		ii
LIST OF FIGURES		iv
LIST OF TABLES		vi
GENERAL INTRODUCTION		vii
1	AMMONIA MONITORING PROGRAM	1
1.1	Introduction	1
1.2	Materials and Methods	2
1.3	Results and Observations	5
1.3.1	Daily Ammonia Patterns	5
1.3.2	Effects of Temperature	8
1.3.3	Effects of Load Rate	8
1.3.4	Effects of Disturbances	13
1.3.5	Mortality	14
1.4	Discussion	15
1.4.1	Ammonia Production under Controlled Conditions	15
1.4.2	Ammonia Production under Hatchery Conditions	18
1.4.3	Chemistry of Aqueous Ammonia	22
1.4.4	Fish Toxicity	23
1.5	Summary	32
2	UREA MONITORING PROGRAM	33
2.1	Introduction	33
2.2	Materials and Methods	34
2.2.1	Urea Analysis	34
2.3	Results and Observations	38

2.4	Discussion	45
2.4.1	Urea Excretion under Controlled Conditions	45
2.4.2	Urea Excretion under Hatchery Conditions	45
2.5	Summary	48
CONCLUSIONS		49
REFERENCES		52
ACKNOWLEDGEMENTS		54
APPENDIX I	ANALYSIS OF OREGON MOIST PELLET	55
APPENDIX II	RELATIONSHIP BETWEEN IONIZED AND UN-IONIZED AMMONIA	55
APPENDIX III	AQUEOUS AMMONIA SOLUTIONS	58
APPENDIX IV	UREA EXCRETION OF FISH	59

LIST OF FIGURES

FIGURES		PAGE
1	DIURNAL AMMONIA PATTERNS OVER A PORTION OF THE STUDY PERIOD.	7
2	GENERALIZED AMMONIA PULSE	5
3	PEAK DAILY AMMONIA CONCENTRATION AS A FUNCTION OF PEAK DAILY TEMPERATURE	9
4	MEAN DAILY AMMONIA PRODUCTION AS A FUNCTION OF MEAN DAILY TEMPERATURE	10
5	MEAN DAILY AMMONIA PRODUCTION OVER THE STUDY PERIOD	11
6	AMMONIA PRODUCTION FOLLOWING THE APRIL 15 POND DISTURBANCE	13
7	PHOTOGRAPH OF FISH AFFLICTED WITH GILL FUNGUS	14
8	DAILY MORTALITY OVER THE STUDY PERIOD	15
9	DIURNAL AMMONIA PULSE AT CONSTANT TEMPERATURE of 59°F	16
10	AMMONIA AND UREA PRODUCTION OF SOCKEYE SALMON UNDER STARVATION CONDITIONS	17
11	PERCENT UN-IONIZED AMMONIA AS A FUNCTION OF pH ($6 \leq pH \leq 13.00$)	24
12	PERCENT UN-IONIZED AMMONIA AS A FUNCTION OF pH ($6.60 \leq pH \leq 8.00$).	25
13	EFFECT OF pH CHANGES ON AMMONIA REDISTRIBUTION	27
14	EFFECT OF AMBIENT AMMONIA ON NITROGEN EXCRETION	31
15	PERCENTAGE ERRORS OF 34 CONTROLS.	37
16	GROUP AVERAGE DIURNAL AMMONIA AND UREA PULSES.	40

17.	AMMONIA AND UREA PRODUCTION OVER THE TEST PERIOD	42
18.	PROPORTION OF UREA AS A FUNCTION OF WATER TEMPERATURE.	43
19.	NITROGEN EXCRETION FOLLOWING A POND DISTURBANCE.	44

LIST OF TABLES.

TABLE		PAGE
1	APPARENT DIFFERENCES BETWEEN THE TWO TEST PERIODS.	4
2	SUMMARY OF AMMONIA PRODUCTION DATA.	6
3	NITROGEN EXCRETION AND ASSIMILATION.	20
4	TRIS BUFFER COMPOSITION.	35
5	GROUPED UREA DATA.	38
6	SUMMARY OF UREA PRODUCTION DATA.	39
7	PROPORTION OF UREA AT VARIOUS LOADING RATES AND TEMPERATURES (Burrows, 1964).	46
8	PROPORTION OF UREA (BIG QUALICUM RESULTS).	46
9	K AND Kw AT DIFFERENT TEMPERATURES.	56
10	PERCENT UN-IONIZED AMMONIA VS. pH AT VARIOUS TEMPERATURES.	57
11	URIC ACID EXCRETION AS COMPARED TO UREA EXCRETION.	60

GENERAL INTRODUCTION

This work is part of a program initiated by the Fisheries Service to investigate certain components of hatchery wastes. Responsibility for the completion of the program was transferred to the Environmental Protection Service, Pacific Region, in April 1972.

The study was conducted at the Big Qualicum Experimental Hatchery between March 9 and October 30, 1972. Two separate programs were carried out during this period. From March 9 to May 6 the ammonia output of a "Burrows Circulating Rearing Pond" loaded with 13 gm. coho fingerlings was intensively monitored. In the body of the report this work is referred to as the "Ammonia Monitoring Program". The second study period, during which ammonia and urea output was monitored, lasted from July 30 to October 30 and involved 6 gm. coho fry. This portion of the work is referred to as the "Urea Monitoring Program". Because the two study periods involve significantly different water temperatures, fish sizes and load rates, separate presentations have been made.

1. AMMONIA MONITORING PROGRAM

1.1 Introduction

Ammonia is a major end product of protein metabolism in fish and so is normally present in waters of salmonid rearing facilities. Concentrations encountered in hatcheries are wide ranging - depending on the size and type of operation. However, ammonia concentrations between 0 and 2.6 mg/ℓ (mean 0.5 mg/ℓ) have been measured at the Cowlitz Trout Hatchery in Washington State (Liao, 1970). This example does not establish typical values, it only serves to point out the range of concentrations encountered at a large production hatchery.

The presence of ammonia is of importance to hatchery operations because it is a cellular poison at relatively low concentrations (Karlson, 1969). At concentrations commonly found in rearing ponds, ammonia irritation can lead to bacterial disease and reduced growth rates (Burrows, 1964). Exposure to higher concentrations of ammonia leads to a multitude of effects. For example, at ammonia levels of 1.0 mg/ℓ severe impairment of the hemoglobin's ability to unite with oxygen occurs (Brockway, 1950).

The purpose of this study was threefold. Firstly, a review of the water chemistry of ammonia was made in order to point out the influence of dissolved oxygen, pH, carbon dioxide and temperature on ammonia toxicity. Such background information is important as ammonia toxicity is very closely related to the chemical nature of the water. For example, a change in pH from 7.0 to 7.5 increases the amount of toxic ammonia present in a given aqueous ammonia solution by approximately three times.

Secondly, the diurnal pattern of ammonia concentration in salmon rearing ponds was also measured. Samples taken randomly over a time period are of little value in characterizing the ammonia concentration in rearing ponds because ammonia, being a metabolic product, varies with the daily activity rhythms of the fish. Diurnal patterns provide a much more realistic indicator of ammonia presence

in rearing ponds.

Thirdly, it was attempted to relate ammonia production to external factors such as pond loading density, water temperature and pond disturbance. It is well known that the oxygen consumption of fish, a measure of metabolic rate, increases markedly with temperature and also during pond cleanings (Elliott, 1969). It would be expected that nitrogen metabolism and resulting ammonia production would also be affected by external factors. Information relating ammonia production with external stimuli would be useful in pre-determining high ammonia situations.

1.2 Materials and Methods

During April, 1971, a 50 foot Burrow's Rectangular Circulating Rearing Pond, located at the Big Qualicum River Experimental Hatchery was loaded with coho salmon fry. By February, 1972, these fish had attained an average weight of 10.8 gms. and the pond load rate had increased to 4.3 lbs. per U.S. g.p.m. However, on March 1 the fish were removed, the pond walls and drain were thoroughly cleaned and the entire system was treated with Malachite Green fungicide. During the next two days nearly 105,000 fish (2,500 lbs.) were ponded, bringing the load rate on March 3 to 6.3 lbs. per U.S. g.p.m.

Ammonia output from the rearing pond was monitored throughout March, April and May, 1972. This program was carried out at the same time as the Big Qualicum Water Treatment Pilot Plant project, so the pond effluent was being analyzed daily for pH, BOD, nitrate etc. A continuous record of water temperatures was also kept.

An automatic sampler (Sigma motor type) was used to draw hourly samples from the rearing pond output well. Samples were fixed with chloroform (1/2 mℓ per 100 mℓ of sample) at the time of collection to allow for ammonia analysis the following day.

Ammonia analysis was carried out by direct Nesslerization. To increase the sensitivity of the test a long cell (10 cm) colorimeter was used to measure absorbance. Although this magnified the effects of turbidity, clarification with zinc sulfate and sodium hydroxide allowed accurate measurement to 0.05 mg/ℓ of ammonia nitrogen.

The monitoring program was carried out from March 15 to May 6. During this period temperatures increased naturally with the onset of Spring. Also, pond load rate increased slowly with the growth of the fish. However, on April 14 enough fish were removed from the pond to significantly reduce the load rate. April 17 to May 6 was termed the Low Load period while March 15 to April 14 was termed the High Load period. The fish removal operation was taken as a typical disturbance routinely experienced by fish under hatchery conditions. The major characteristics of the two periods are summarized in Table 1.

TABLE 1. APPARENT DIFFERENCES BETWEEN THE TWO TEST PERIODS.

Parameter	Low Load Rate Period	High Load Rate Period
Period	April 17 to May 6	March 15 to April 14.
Pond Volume	1,697.5 ft^3; 48,068.1ℓ	1,697.5 ft^3; 48,068.1ℓ.
Flow through Pond.	400 U.S. g.p.m. 90,840 ℓ per hr.	400 U.S. g.p.m. 90,840 ℓ per hr.
Mean Daily Temperature	44.6°F; 7.0°C Range 42.2 to 48.0°F	41.2°F; 5.1°C Range 40.0 to 43.5°F
Average Feed Rate	1.97% Range 1.73% to 2.30%	1.20% Range 0.89% to 1.50%
Feeding	1/8 inch Oregon Moist Pellets automatically fed every 30 minutes from dawn until dusk.	1/8 inch Oregon Moist Pellets (1) automatically fed every 30 minutes from dawn until dusk.
Mean Fish Size	15.3 gms Range 14.0 to 16.5 gms	12.1 gms Range 11.5 to 13.9 gms.
Mean Load Rate	5.4 lbs. per U.S. g.p.m. Range 5.2 to 5.5	7.1 lbs. per U.S. g.p.m. Range 6.6 to 8.0
Mean Load Density	1.27 lbs per ft^3 Range 1.23 to 1.30	1.69 lbs. per ft^3 Range 1.55 to 1.88.

(1) See Appendix 1.

1.3 Results and Observations.

1.3.1 Ammonia Patterns. Daily ammonia nitrogen production rates have been listed in Table 2. All daily mean ammonia concentrations were calculated from complete diurnal samples. Days on which sampling was carried out over the afternoon period only were omitted from Table 2. Daily food rations have also been shown. The nitrogen input to the pond was calculated from the fact that the food contained 35% protein (wet weight) and that the protein was 16% nitrogen by weight. The percentage of the protein nitrogen ration excreted as ammonia was calculated from:

$$\frac{\text{Grams of nitrogen excretion as ammonia per day}}{\text{Grams of nitrogen fed per day}} \times 100\%$$

Typical diurnal variations in the ammonia concentration of the rearing pond effluent are shown in Fig. 1. The ammonia was at a minimum concentration during mid morning (0300 to 0800) and reached a maximum in the afternoon (1300 to 1900). The ammonia peak coincided approximately with the daily temperature peak. These peaks were noticed to occur about 9 hours after the automatic feeders began operation. A generlized ammonia pulse is shown in Figure 2.

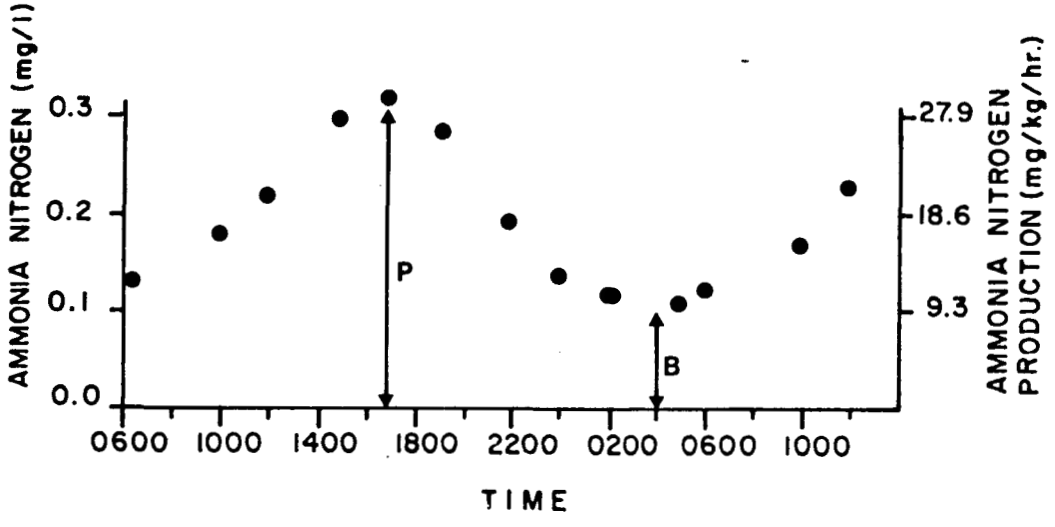

FIGURE 2: GENERALIZED AMMONIA PULSE

TABLE 2. SUMMARY OF AMMONIA PRODUCTION DATA

Date	Period	Ammonia Nitrogen Production			Ration		Percentage of daily N. ration excreted as NH_3	Mean Temp ^{o}F
		Mean conc.(mg/ℓ	Weight (gms)	Rate(mg/kg/hr)	Total wet weight	Nitrogen (gms)		
March (9-10)	1630-1630	0.17 mg/ℓ	371	13.1 mg/kg/hr	20 lbs.	509	73%	38.6±0.3
March (12-13)	" "	0.11	240	8.4	20	509	47	39.5
March (15-16)	" "	0.17	371	12.9	20	509	73	41.2
March (18-19)	" "	0.18	392	13.5	20	509	77	40.1
March (23-24)	" "	0.15	327	11.0	21	529	62	40.2
March (27-28)	" "	0.18	392	12.9	21	529	74	40.9
March (29-30)	1030-0830	0.17	371	12.1	30	750	49	41.6
April (2-3)	1630-1430	0.24	523	16.6	36	925	57	40.9
April (15-16)	1000-0800	0.28	610	27.1	30	763	80	42.8
April (17-18)	1130-0930	0.19	414	18.2	30	763	54	42.3
April (20-21)	0600-0600	0.153	334	14.2	36	915	30	43.1
April (22-23)	" "	0.126	275	11.5	36	915	34	42.8
April (23-24)	" "	0.141	307	12.8	36	915	28	43.5
April (26-27)	" "	0.156	340	15.0	48	1220	30	43.9
April (27-28)	" "	0.169	368	16.1	48	1220	30	45.5
April (28-29)	" "	0.165	360	15.6	48	1220	30	44.7
April (29-30)	" "	0.165	360	15.5	48	1220	30	44.0
May (3-4)	" "	0.165	360	15.1	48	1220	30	46.2
May (4-5)	" "	0.204	445	18.6	48	1220	36	47.2
May (5-6)	" "	0.190	414	17.3	48	1220	34	47.8

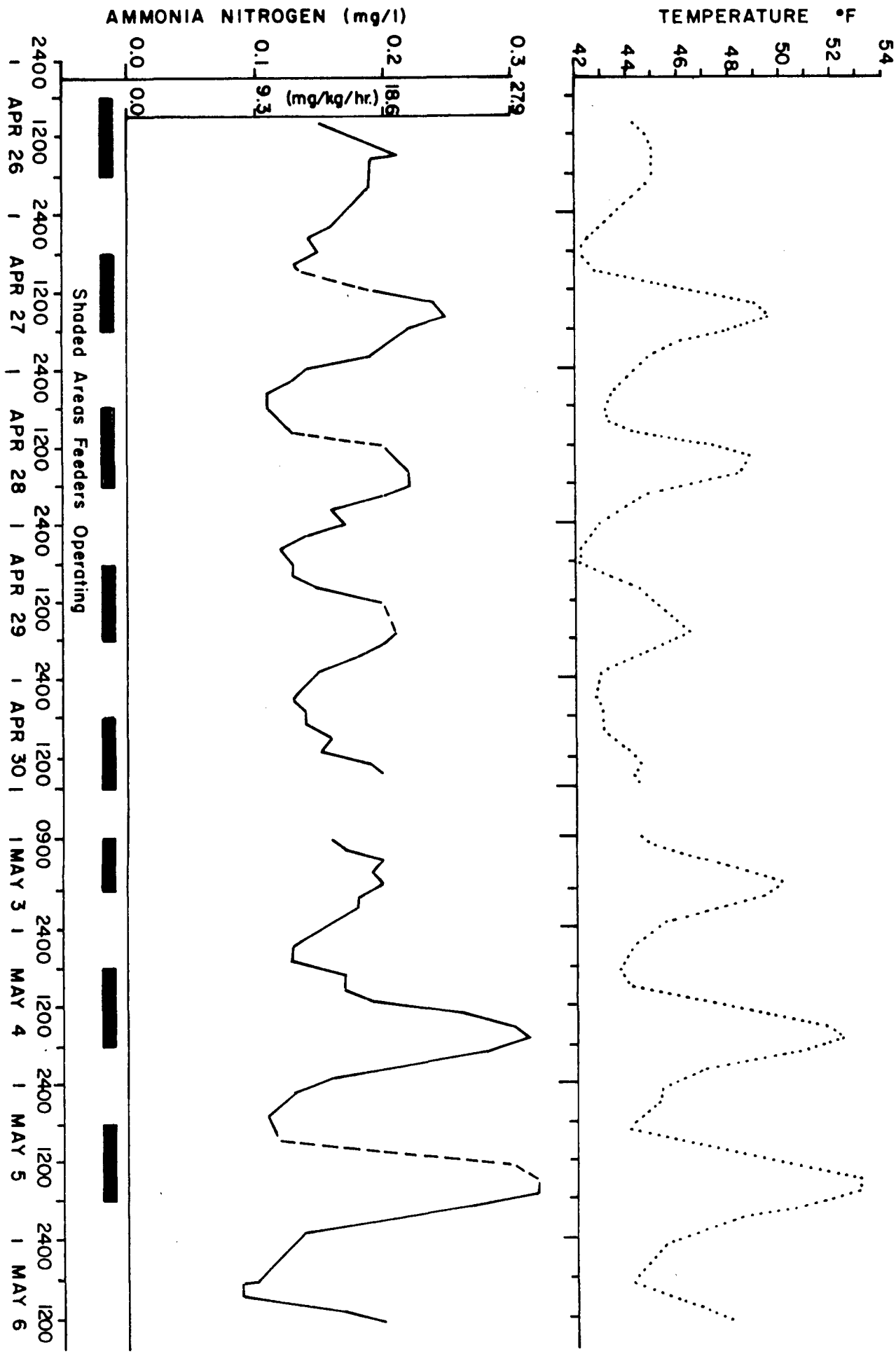

FIGURE 1. DIURNAL AMMONIA PATTERNS OVER A PORTION OF THE STUDY PERIOD.

1.3.2 The Effects of Temperature on Ammonia Production. Peak daily ammonia values recorded over the low load period have been plotted against peak daily temperatures (Fig. 3). The pattern is described by the least square regression line as:

$Y = -22.5 \times 10^{-5} + 0.7 \times 10^{-5} T$, Correlation coefficient = 0.95

where Y = peak daily ammonia level in mg/ℓ per pound of fish.

T = peak daily temperature (° F)

or $Y = -45.1 + 1.4T$

where Y = peak daily ammonia production mg/kg/hr

T = peak daily temperature (° C)

Date point (1) Figure 3 was taken as atypical and not included in the calculation of the regression line. On this particular day the hatchery crew noticed that the fish were not feeding. Apparently this inacitivity was reflected in the daily ammonia pulse.

The average daily ammonia output has also been plotted against temperature (Fig. 4). However, temperature in this case represents the mean daily temperature. The least squared regression line over the low load period has been calculated to be:

$W = 1.0Z - 29.5$. correlation coefficient = 0.85

where W = mean daily ammonia production in mg/kg/hr.

and Z = mean daily temperature (°F).

1.3.3 The Effect of Pond Load Rate on Ammonia Output. The daily ammonia production (mg/kr/hr) has been plotted against time (Fig. 5). Ammonia production over the "High" load period (March 15-April 14) averaged 14.5 mg/kg/hr. This was comparable to an average production of 15.0 mg/kg/hr measured over the "Low" load period. If water temperatures were constant over the two test

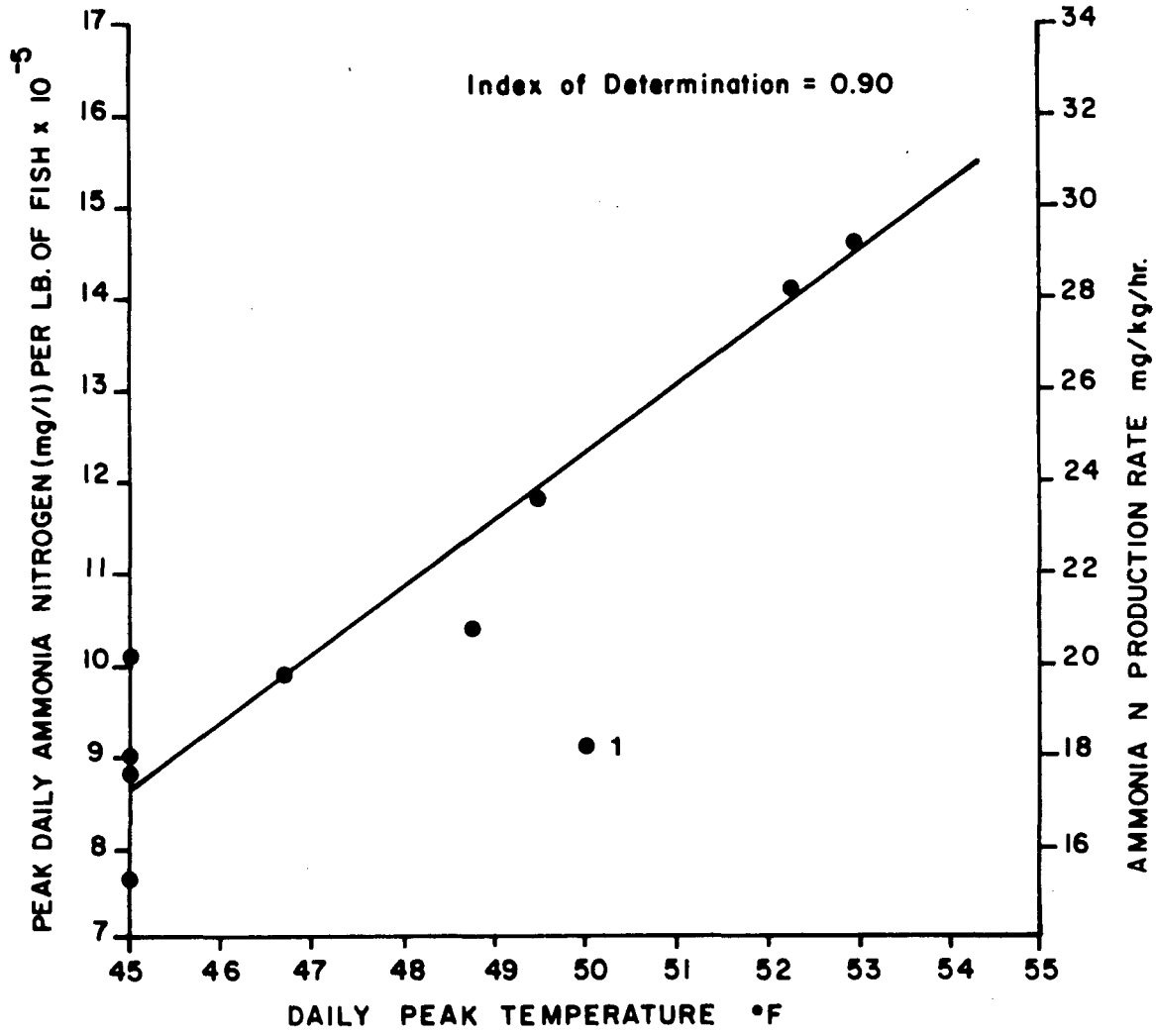

FIGURE 3: PEAK DAILY AMMONIA CONCENTRATION AS A FUNCTION OF PEAK DAILY TEMPERATURE.

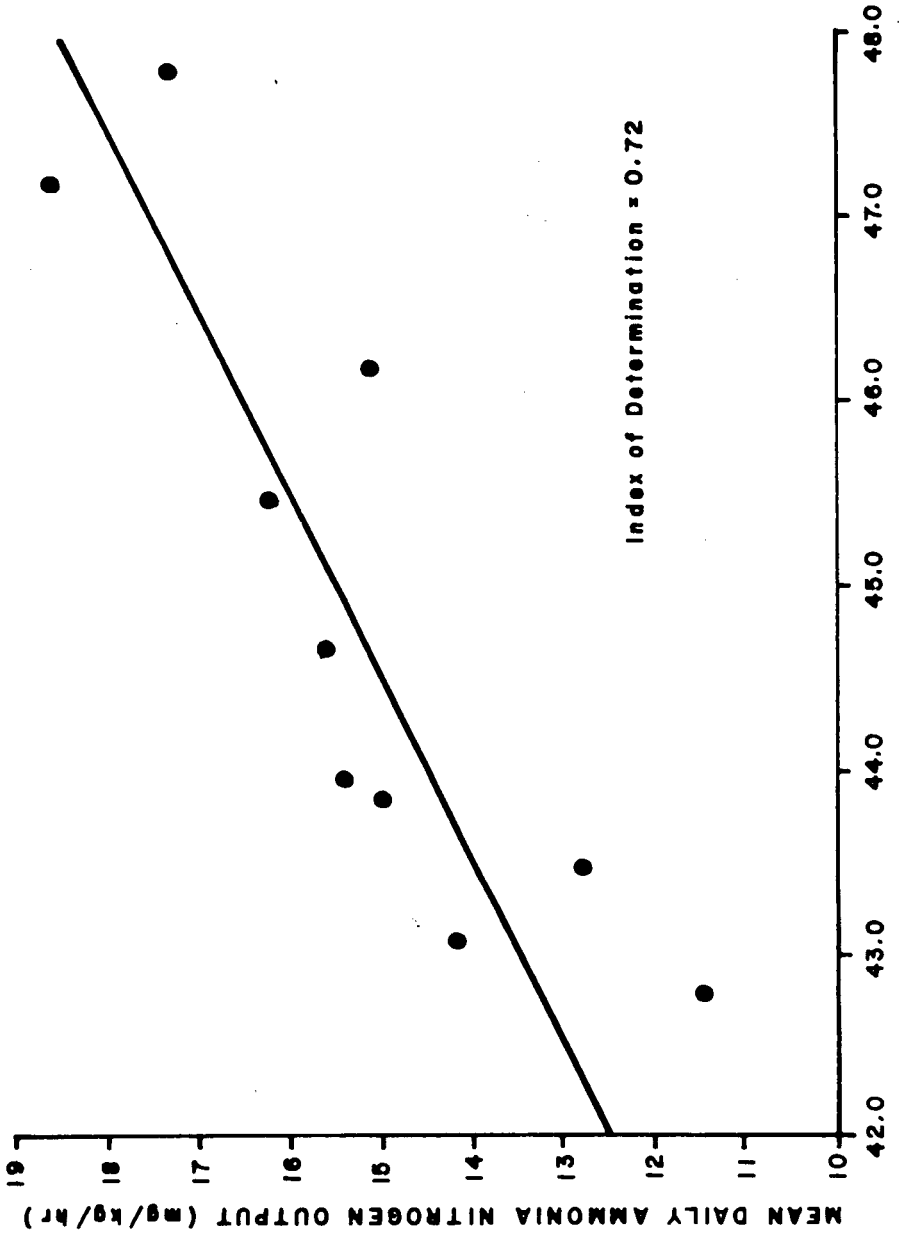

FIGURE 4: MEAN DAILY AMMONIA PRODUCTION AS A FUNCTION OF MEAN DAILY TEMPERATURE.

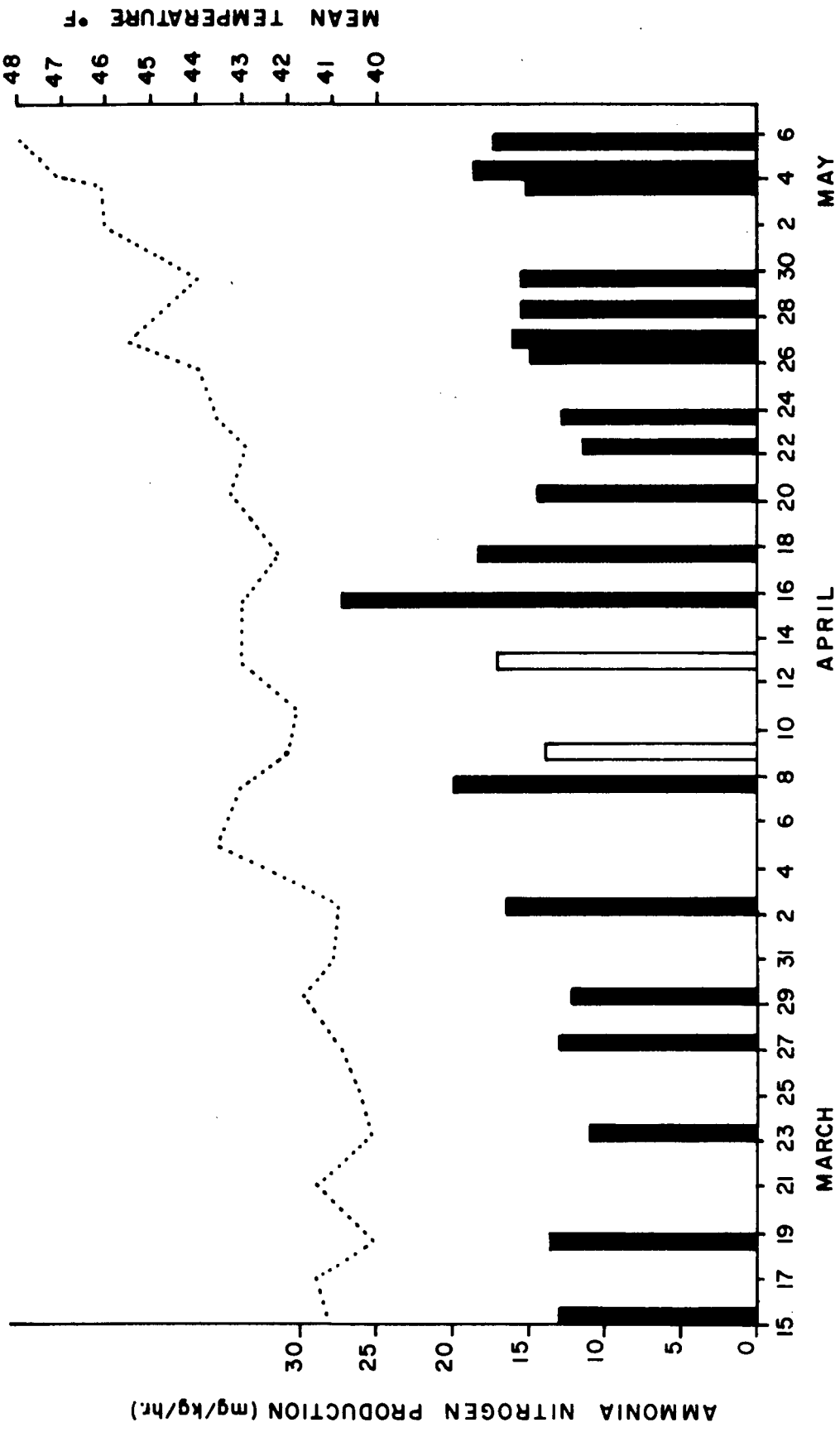

FIGURE 5: MEAN DAILY AMMONIA PERIOD OVER THE STUDY PERIOD. LIGHTLY SHADED BARS INDICATE DAYS WHEN INCOMPLETE DIURNAL SAMPLES WERE TAKEN.

periods it could be concluded from these figures that ammonia production was independent of loading rate.

However, the mean daily water temperature during the "High" load period averaged only 41.2°F - this was 3.4°F less than the mean water temperatures encountered during the "Low" load period. Assuming ammonia production to be independent of loading rate, the expected output at the lower temperature could be calculated from the regression line relating mean daily ammonia production to temperature. (Fig. 4).

$$W = 1.0Z - 29.5$$

Where Z = temperature °F

W = ammonia production.

Substituting Z = 41.2°F into this expression allowed calculation of the expected output, "W".

$$W = 11.7 \text{ mg/kg/hr.}$$

On this basis, the ratio of ammonia production over the two load rates should have been:

$$\frac{\text{"Low" load ammonia production}}{\text{"High" load ammonia production}} = \frac{15.0 \text{ mg/kg/hr}}{11.7 \text{ mg/kg/hr}} = \simeq 1.28$$

On the other hand, actual measurement showed that ammonia production over the "High" load period was greater than expected, such that:

$$\frac{\text{"Low" load ammonia production}}{\text{"High" load ammonia production}} = \frac{15.0 \text{ mg/kg/hr}}{14.5 \text{ mg/kg/hr}} = \simeq 1.03$$

The measured value of 14.5 mg/kg/hr was outside the 90% confidence interval for the predicted value of ammonia production at 41.2°F.

1.3.4 The Effect of Disturbance on Pond Ammonia Levels. On April 14 screens were placed in the pond and 24,000 coho (720 lbs.) were removed. At 0900, April 15, screens were again placed in the pond and 390 lbs. of fish were removed. About 2,000 lbs. of fish (66,270) remained in the pond. The auto sampler was put into operation at 1000, April 15. The diurnal ammonia pulses for April 15-16, April 17-18, and April 22-23 were recorded (Fig. 6).

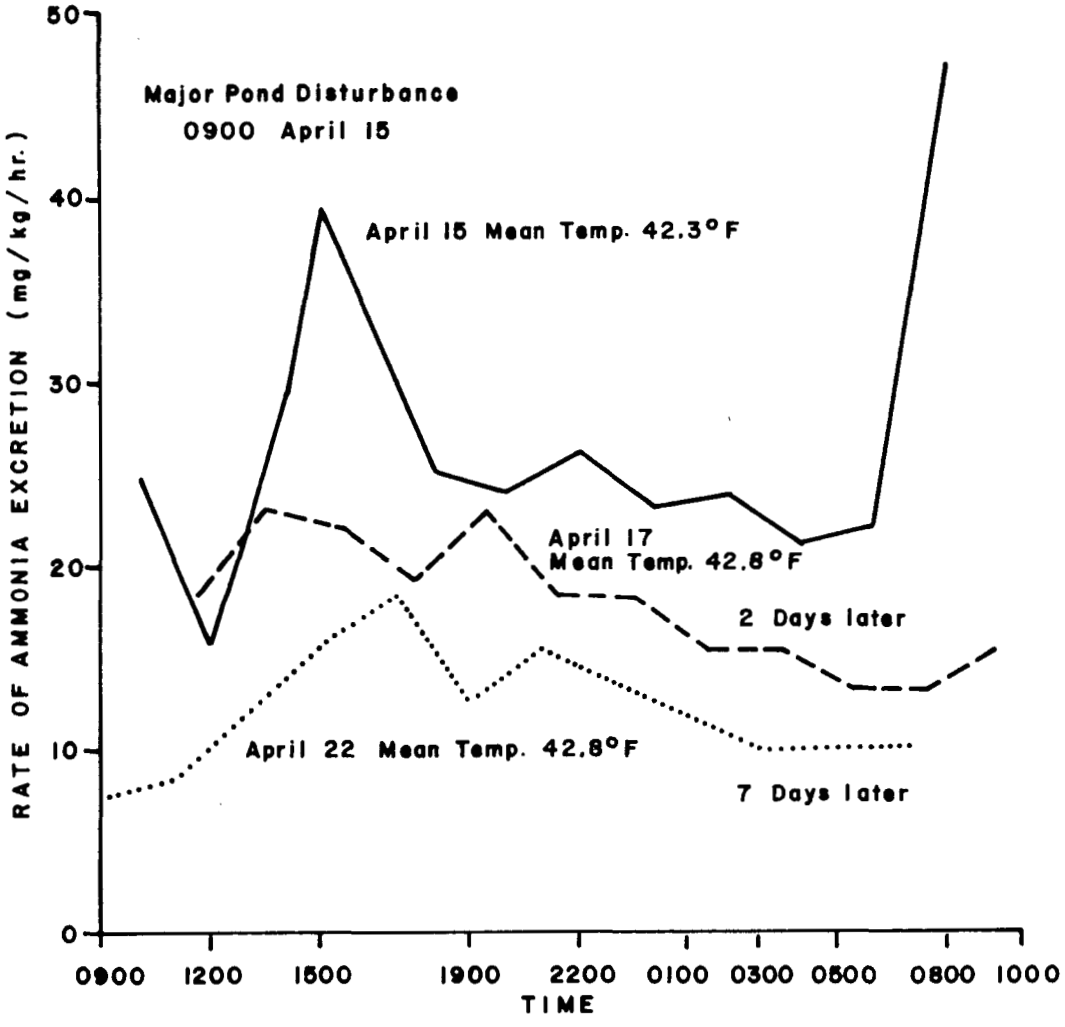

FIGURE 6. AMMONIA PRODUCTION FOLLOWING THE APRIL 15 POND DISTURBANCE.

The April 15 pulse showed a value of 0.3 mg/ℓ at 1000; this was probably an immediate response to the disturbance. The effect of this disturbance however, was long lasting - values of ammonia continued to be high (0.24 mg/ℓ) throughout the night.

Ammonia values normally begin to rise slowly at about 0600. The morning of April 16 however saw a very dramatic rise - the concentration of ammonia rose to 0.48 mg/ℓ in less than 2 hours. Two days later, (April 17) ammonia levels were still higher than would be expected at the given temperature and loading rate.

1.3.5 The Observed Effect of "High" Loading Rates on Rearing Pond Coho. Recorded daily mortalities were graphed over the study period (Fig. 8). During the "High Load" period mortality rose from 0.22% to 0.32% per day. However, much of this increase took place in the two weeks following the March pond load increase and so could have been the result of handling.

Throughout the first two weeks of April noticeable numbers of fish had gill fungus (Fig. 7). This condition was observed during the period of highest load when the ammonia concentrations were at a maximum. However, during this period mortality remained low at about 0.010% per day.

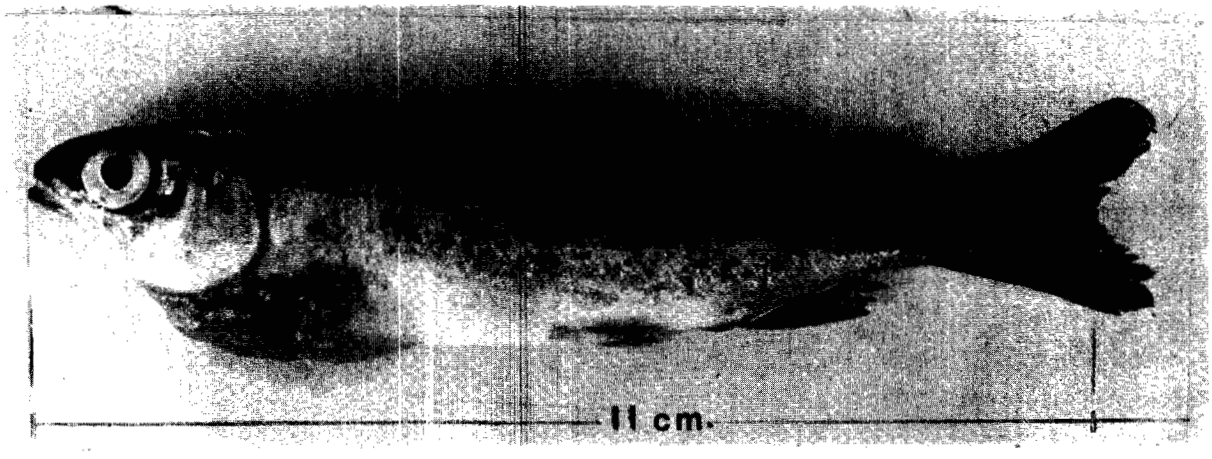

FIGURE 7. PHOTOGRAPH OF FISH AFFLICTED WITH GILL FUNGUS.

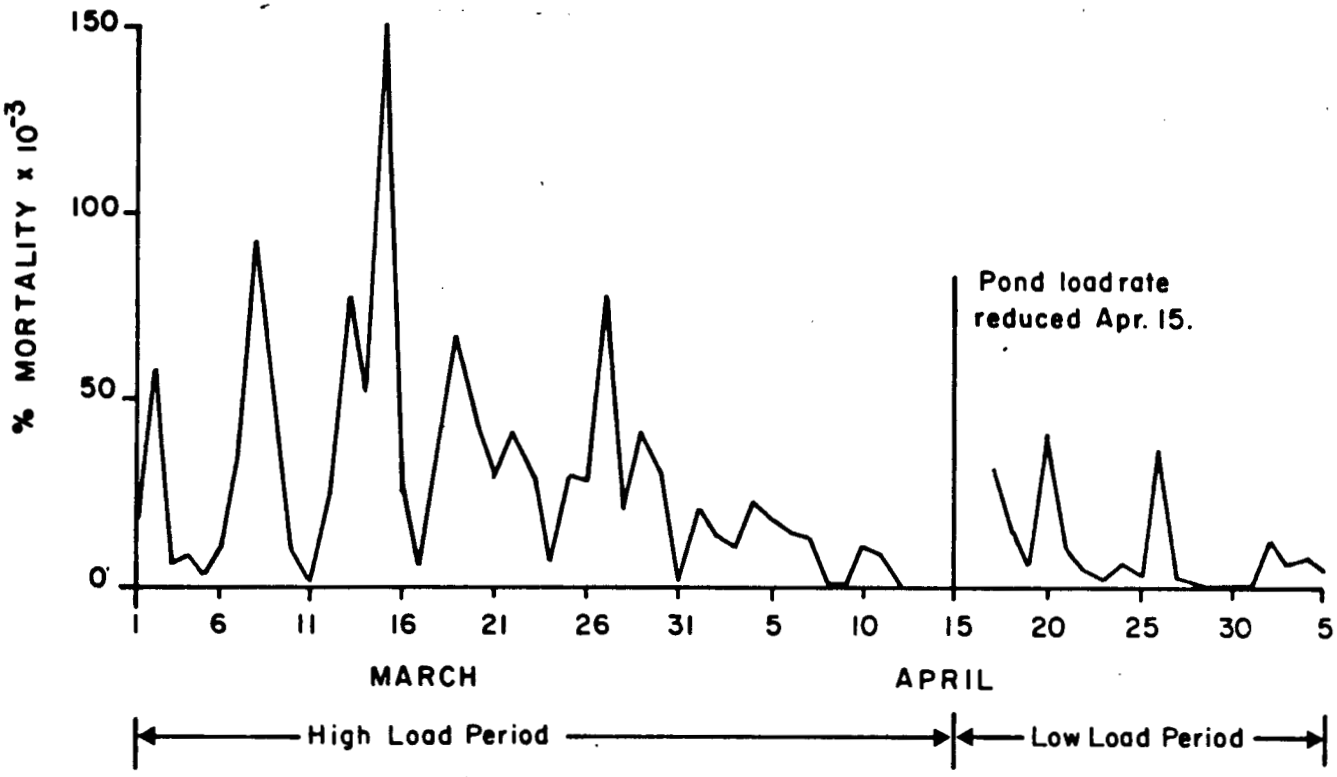

FIGURE 8: RECORDED DAILY MORTALITY (AS PERCENT) OVER THE STUDY PERIOD.

1.4 Discussion

The diurnal ammonia pulses observed over the test period (Fig. 1) have been affected by many variables. In the hatchery environment, photoperiod, time and duration of feeding, temperature and stress situations vary daily. These factors alter metabolic rates and hence ammonia production.

1.4.1 Ammonia Production under Controlled Conditions. Some insight can be gained into the influence of these variables by measuring diurnal ammonia production under controlled conditions. Results of work carried out with Sockeye Salmon under laboratory conditions have been graphed in Fig. 2. (2)

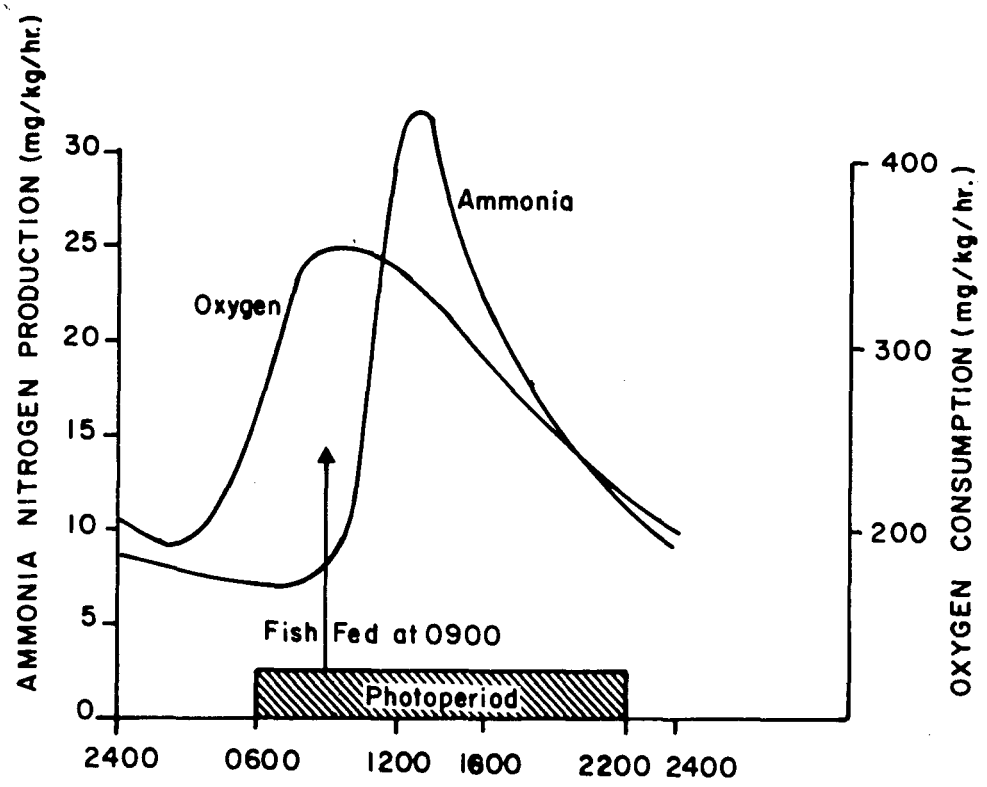

FIGURE 9. DIURNAL AMMONIA, OXYGEN AND UREA PULSES AT A CONSTANT TEMPERATURE OF 15°C, (59°F). (2)

During this experiment temperature was held constant at 59°F, the photoperiod was controlled and the fish were fed a maintenance ration at 0900 every morning. Under these conditions the metabolic rate (oxygen consumption) was stimulated by first light but peaked during feeding. Ammonia excretion on the other hand, was stimulated by feeding reaching a peak about four hours after feeding began.

(2) These measurements are shown, courtesy of Dr. J.R. Brett, Data was obtained from experiments carried out by the Fisheries Research Board of Canada at the Nanaimo Biological Station. A complete account of this work will be published at a future date.

The nitrogen excretion patterns of starved fish were also studied (Fig. 10) [2].

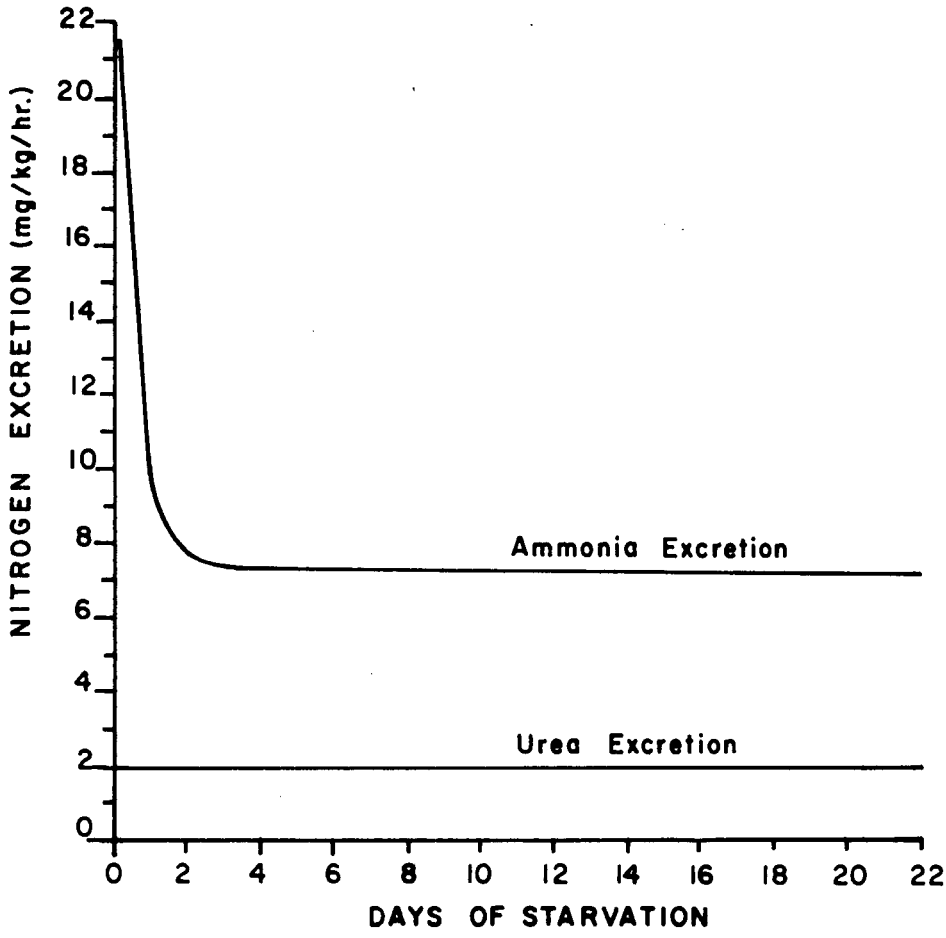

FIGURE 10: AMMONIA AND UREA PRODUCTION OF SOCKEYE SALMON UNDER STARVATION CONDITIONS.

As before, water temperature was held constant and the photoperiod was regulated. Under these conditions no diurnal variation was measured. After two days of starvation the nitrogen excretion rate fell to a relatively constant level of 9.2 mg/kg/hr. This value was taken to approximate the endogenous nitrogen excretion of sockeye.

Endogenous nitrogen excretion corresponds to the minimum intake

of protein required for maintenance. Protein intake must exceed this rate of nitrogen excretion for growth to occur (Gerking, 1955).

Endogenous nitrogen measurements become significant when they are compared to the metabolic rate of the animal under study. For example, warm blooded animals generally excrete about 2 mg. of nitrogen per basal Kilocalorie (Maynard, 1951). On the other hand it was calculated that the endogenous nitrogen excretion of sockeye was about 16 mg. per Kilocalorie. Furthermore, Gerking (1955) reported that the Bluegill Sunfish excreted about three and a half times more nitrogen than warm blooded animals per basal Kilocalorie. Such measurements suggest that fish rely heavily on protein catabolism for energy. The high rates of ammonia nitrogen production associated with salmon rearing facilities are better appreciated in light of this fact.

1.4.2 Ammonia Production under Hatchery Conditions. Important differences exist between the controlled conditions of the sockeye experiments and the hatchery conditions under which salmon are normally reared. Under hatchery conditions, feed rates are held above maintenance levels; the fish have the opportunity to feed over the entire photoperiod; temperatures vary throughout the day and growth is taking place. All these factors modify ammonia production.

Because hatchery reared fish can feed any time over the photoperiod, ammonia production is more evenly distributed throughout the day (Fig. 2). Also, rearing pond water temperatures are seldom constant. Afternoon water temperature surges would increase metabolic rates and further modify ammonia output. The observed correlation between daily ammonia production and temperature (Fig. 3 and Fig. 4) results from the influence of temperature on metabolic rates and feeding intensity.

Nitrogen excretion would also be influenced by the rate of growth. This is best understood by considering the protein nitrogen budget of the rearing pond. The total daily nitrogen ration is equal to the sum of the nitrogen assimilated as growth, the nitrogen excreted

and the nitrogen lost directly as waste.

It follows that for a given ration, increased growth rates would cause decreases in the production of ammonia.

Although this is a theoretical relationship it has relevance to hatchery operation. Accurate measurement of growth rates would allow an estimation of the rate of protein nitrogen assimilation. Diurnal measurements of ammonia and urea nitrogen would account for most of the protein catabolized for energy.

For example, bi-weekly growth rate measurements showed that between March 9 and May 6 a total ration of 2,100 lbs. of food resulted in 868 lbs. of growth. In other words 53,390 gm of protein nitrogen resulted in the assimilation of 9,458 gm. of nitrogen as protein (assuming growth to be 15% protein). Twenty-one diurnal ammonia samples taken over this period showed that on the average, between 45 and 55% of the nitrogen ration was excreted as ammonia nitrogen. Therefore, 18% of the total nitrogen ration was assimilated in growth and approximately 50% was excreted as ammonia. Urea nitrogen and waste food would probably account for the remaining 32%.

Table 3 shows estimations of the nitrogen excreted and assimilated over three different rearing pond environments. Nitrogen excretion represents a minimum figure, as urea nitrogen production was not measured. Also, the amount of food lost directly as waste was not accounted for.

Low temperature conditions (T = 38.6°F) resulted in lower nitrogen intake (364 mgN per Kg. as opposed to 646 mgN per Kg at 47.2°F), also a lower percentage of the digested protein was assimilated as growth (14% as opposed to 30% at 47.2°F). It was noted that the nitrogen excreted on April 15 accounted for 80% of the total protein nitrogen fed during the day. Since the nitrogen excretion value is a minimum figure, it would be safe to assume that during this period of constant disturbance, the fish were probably in negative nitrogen balance.

TABLE 3. AN ESTIMATION OF NITROGEN EXCRETION AND ASSIMILATION OVER THREE WIDELY VARYING SETS OF CONDITIONS.

Rearing Pond Conditions	Minimum nitrogen excreted per day	Estimated nitrogen assimilated in growth per day	Protein nitrogen ration
March 9 Normal operation Temp. = 38.6°F.	314 mgN/Kg.	50 mgN/Kg	432 mgN/Kg
April 15 Fish undergoing disturbance Temp. = 42.3°F	594 mgN/Kg	∿ 0	763 mgN/Kg.
May 4 Normal operation Temp. = 47.2°F.	446 mgN/Kg	200 mgN/Kg	1220 mgN/Kg

Ammonia excretion during the period of disturbance was 25 mg/kg/hr. This compared with an average value of 14 mg/kg/hr measured under normal hatchery conditions. This increase might be the direct result of increased metabolic rates. Since protein is an important energy source for fish, ammonia production would increase in direct proportion to the metabolic rate.

However, recent work has shown that under certain conditions, ammonia production increases at a much faster rate than would be expected by measurement of increases in the metabolic rate. Kutty, (1972) found that during periods of low ambient dissolved oxygen or during forced activity, fish rely more heavily on protein as a fuel source. At low ambient dissolved oxygen Kutty found that the ammonia quotient [3] increased fivefold. Also, during forced activity the ammonia quotient showed a fourfold increase

(3) Ammonia quotient = $\frac{\text{Volume of } NH_3 \text{ formed}}{\text{Volume of } O_2 \text{ consumed}}$

from the 1st hour value to that of the 6th hour of exercise. This was taken to suggest an increased utilization of protein.

Kutty believes that the reason for this switch to protein metabolism and hence increased ammonia excretion might possibly lie in the maintenance of blood pH. A fish under forced activity, quickly passes into anaerobic energy utilization. The lactic acid produced as the end product of glycolysis lowers blood pH. However, switching to protein metabolism as a energy source tends to counter the effects of lactic acid. It may be that anaerobic energy utilization and protein metabolism are coupled. In light of this possibility the increased ammonia excretion observed during rearing pond disturbances (pond cleanings, transplants etc.) is readily understandable.

The data taken over the "High" load rate period suggested that ammonia production was stimulated to some degree. Approximate calculations showed that the average production was about 30% greater than would be expected on the basis of the ammonia-temperature regression line. However, this figure does not quantify the effects of pond loading rate on ammonia production as several pertinent variables were not constant over the test periods. Correction factors for feed rate, fish size and growth rate differences were not included in the comparison of ammonia production over the two load rates.

The basis of this hypothesized relationship might lie in the effects of crowding on the metabolism of the fish. High load or high density conditions might cause excitation due to crowding. Increased metabolic rates lead, in direct proportion, to increases in ammonia production.

Alternately, stress conditions induced by high densities might stimulate disproportionate increases in protein catabolism. This results in increased ammonia production without a corresponding increase in the metabolic rate. It should be emphasized that any factor that stimulates disproportionate nitrogen excretion, decreases the amount of nitrogen available for growth.

It should be pointed out that the ammonia concentrations of the rearing pond input water were not measured on a diurnal basis. Therefore, pond ammonia production could not be completely equated with the protein catabolism of the fish. However, six measurements over the test period showed that the ammonia concentration of the input water was very low (less than 0.03 mg/ℓ). It was assumed that on the average, input water contributed less than 10% of the ammonia production of the rearing pond.

It was thought that uneaten fish food might decompose in the pond and thereby contribute to the pond ammonia output. High concentrations of fish food mixed with pond water, however showed that the contribution of the uneaten food would be negligible, considering the flushing rate of the rearing pond.

The possibility that bacterial action on excreta might alter ammonia and urea concentrations over time, has been investigated by Wood (1958). It was reported that during a 24 hour period little change occurred in the ammonia and urea concentration of waste water held at 12°C. On this basis, only accumulations of waste food and excreta would be expected to affect rearing pond ammonia and urea production. However, the contribution of wastes collected on the pond bottom and in the drain was not assessed.

1.4.3 Chemistry of Aqueous Ammonia. Regardless of how ammonia is introduced into the water, it is generally considered a toxic substance to fish. A review of the water chemistry of ammonia is useful in appreciating some of its effects.

Aqueous ammonia exists in the following equilibrium (see Appendix 2):

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

A solution of ammonia in water therefore contains un-ionized ammonia NH_3, and ionized ammonia, NH_4^+. The Nessler's test for ammonia measures both the ionized and un-ionized forms.

1.4.4 Fish Toxicity. It is widely accepted that the un-ionized ammonia (NH_3) is toxic to fish whereas the ionized ammonia (NH_4) is relatively harmless. Un-ionized ammonia is much more toxic than ionized ammonia because it diffuses through biological membranes readily due to its lack of electrical charge and high lipid solubility. (Fromm and Gillette, 1968).

The relationship between ionized ammonia and un-ionized ammonia is a function of both pH and temperature. It can be shown (Appendix 2) that as pH and temperature increase, the percentage of un-ionized ammonia increases markedly. Plots have been made of percent un-ionized ammonia vs. pH at various temperatures (Fig. 11). Fig. 12 shows a graph of percent un-ionized ammonia over the range of pH values normally encountered in salmon rearing ponds.

In considering the effect of un-ionized ammonia on fish its relationship to other chemicals in the water must be kept in mind. Fixed acids such as HCl lower the toxicity of a given concentration of ammonia. This can be understood by considering the distribution of ammonia across a hypothetical biological membrane (Fig. 13a). The passage of ionized ammonia through the membrane is inhibited whereas un-ionized ammonia, depending on the concentration gradient, moves freely. When an acid such as HCl is added to the water, the environmental pH is lowered (Fig. 13b). More importantly an un-ionized ammonia concentration gradient is created across the membrane. This speeds the passage of ammonia out of the fish and effectively traps it in the environment as ionized ammonia.

Increasing the dissolved carbon dioxide content of the water, although lowering the pH, does not lower ammonia toxicity. From work carried out with rainbow trout Lloyd and Herbert (1960) found that ammonia toxicity actually increased when the carbon dioxide concentration was increased from 3.2 mg/ℓ to 48 mg/ℓ (pH dropped from 8.2 to 7.0). Warren and Schenker (1962) feel that carbon dioxide, unlike fixed acid, permeates the membrane

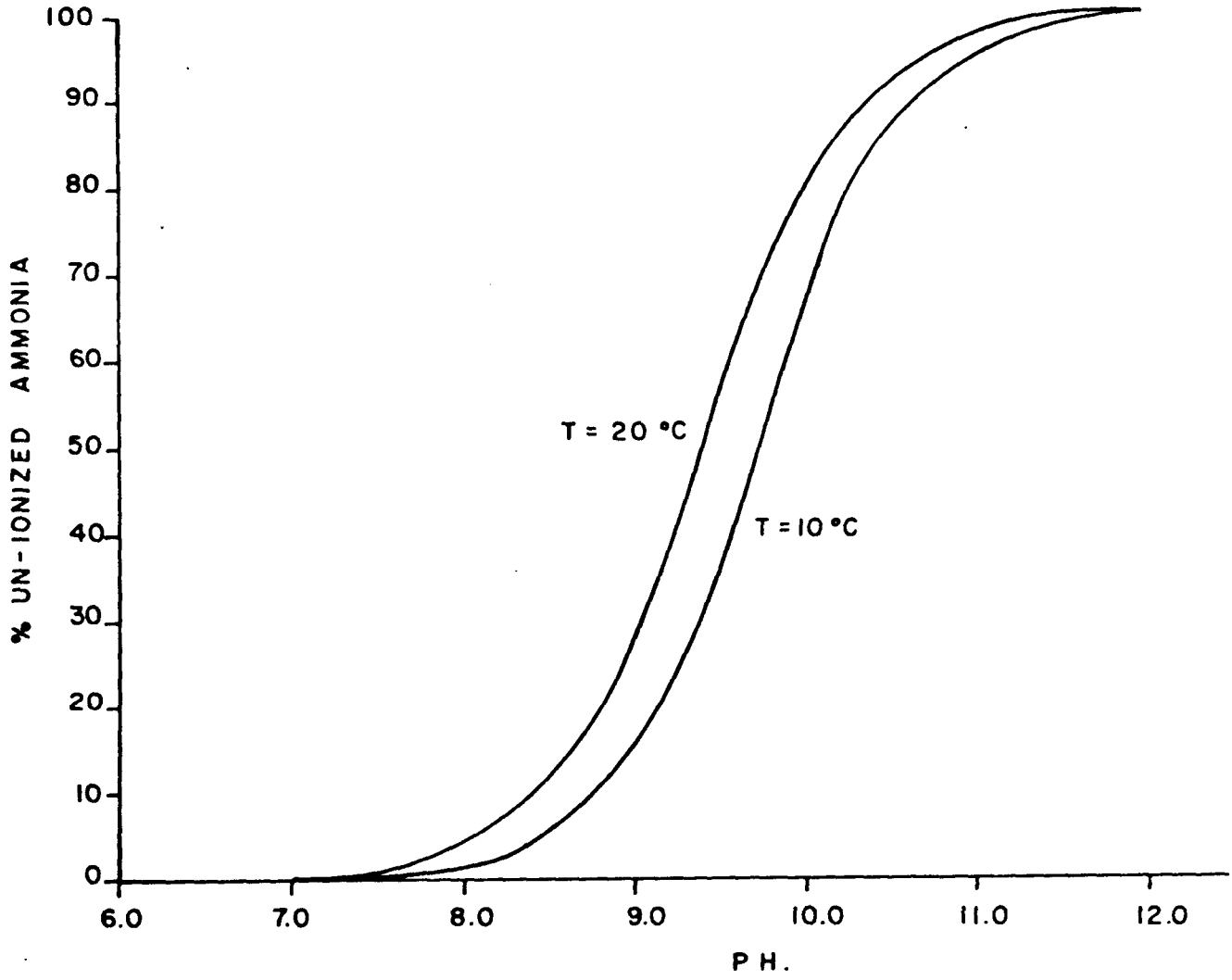

FIGURE 11. PERCENT UN-IONIZED AMMONIA AS A FUNCTION OF pH (6 < pH < 13).

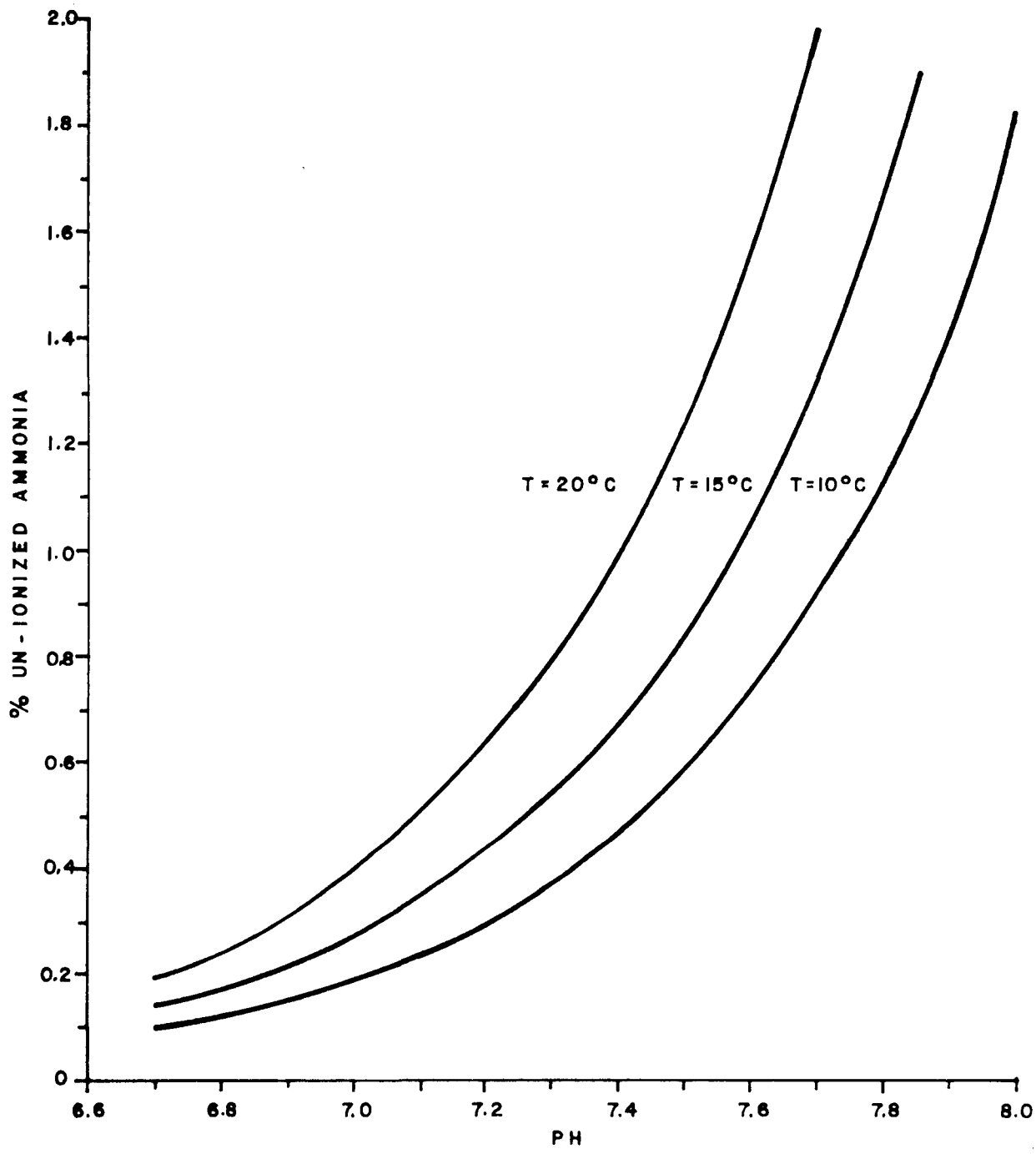

FIGURE 12: PERCENT UN-IONIZED AMMONIA AS A FUNCTION OF pH
(6.60 < pH < 8.00)

thereby destroying the pH gradient necessary for ammonia redistribution (Fig. 13c).

The toxicity of ammonia has also been found to increase at low concentrations of dissolved oxygen. A concentration of un-ionized ammonia of 0.02 mg/ℓ was found to decrease the survival time of trout at a level of dissolved oxygen that otherwise had no lethal effect (McKee and Wolf, 1971).

Burrows (1964) believes that un-ionized ammonia irritation is the precursor of bacterial gill disease. High levels of un-ionized ammonia cause gill irritation that allows bacterial infection to occur. Bacteria interfere with normal gas exchange causing the cells of the gill epithelium to multiply - this process eventually leads to complete fusion of the gill filaments. Gills in this conditions are very prone to fungus invasion.

Gill fungus was observed to develop during the first two weeks of April when both pond loading rate and pond ammonia levels were at a maximum. Whether the gill fungus developed as a result of high ammonia levels or not is difficult to ascertain; however, it is interesting to relate Burrow's observations concerning bacterial gill disease to the coho under study.

From work carried out with Chinook fingerlings at a temperature of 15°C and pH 7.8 it was concluded that continuous exposure for 6 weeks to concentrations of ammonia as low as 0.3 mg/ℓ could cause extensive hyerplasia of the gill epithelium (Burrows, 1964). The concentration of un-ionized ammonia present could be calculated from equation V (Appendix 2):

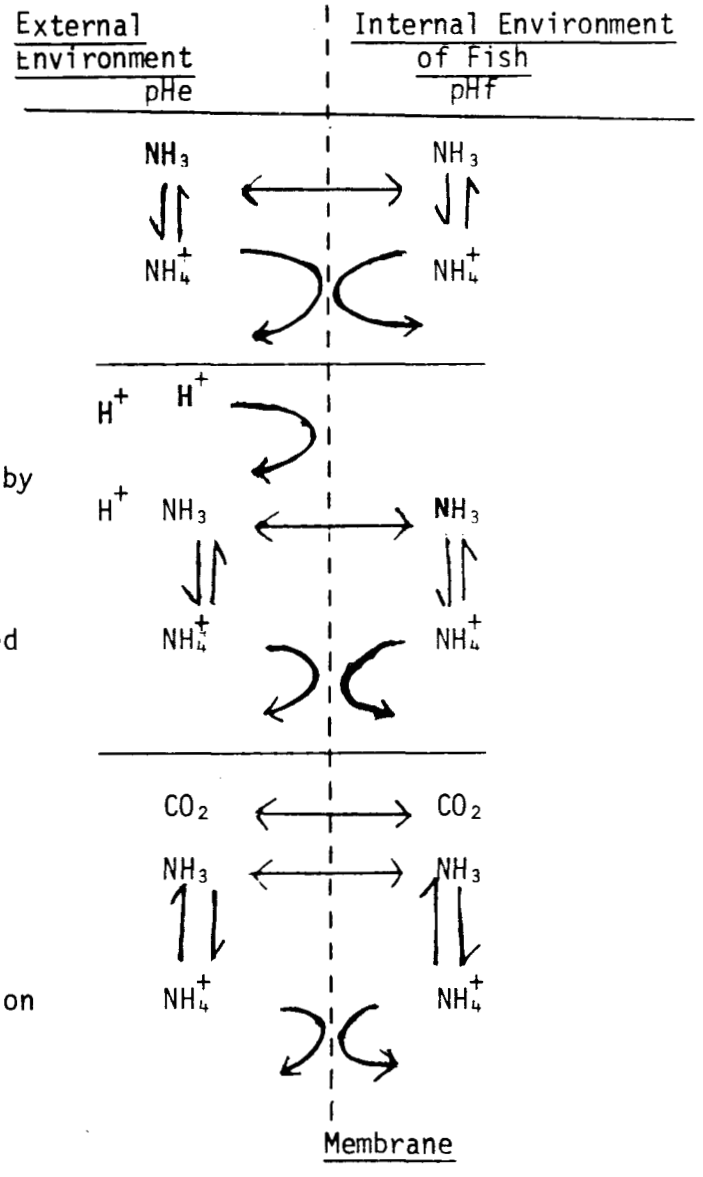

Fig. 13 (a)

No pH gradient:
pHe = pHf.

Fig. 13 (b)

pH gradient created by the addition of HCl
pHe<pHf

Result: Ammonia redistributed

Fig. 13 (c)

Addition of carbon-dioxide

Result: Ammonia redistribution less marked

FIGURE 13: EFFECT OF pH CHANGES ON AMMONIA REDISTRIBUTION

$$\frac{[NH_3]}{A} = \frac{1}{1 + \frac{K}{Kw\ 10^{pH}}}$$

where: $[NH_3]$ = unknown
A = 0.3 mg/ℓ
K = 1.652×10^{-5}
$-\log Kw$ = 14.3463
pH = 7.8

Substituting these values into equation V and solving for NH_3 gives, NH_3 = 0.0051 mg/ℓ. The mean water temperature at Big Qualicum (April) was 10°C while the maximum pH recorded was 7.6 The ammonia concentration (un-ionized + ionized) required to produce 0.0051 mg/ℓ of un-ionized ammonia at these conditions was found to be 0.44 mg/ℓ (from formula V). At Big Qualicum, measurable ammonia levels would have to be at least 0.44 mg/ℓ before un-ionized ammonia concentrations reached those capable of causing gill hyperplasia. At no time during the Spring program were ammonia concentrations of 0.44 mg/ℓ encountered.

It is very difficult to literally apply Burrow's results to the rearing pond environment. Firstly, ammonia levels could have been much higher in certain areas of the pond than was indicated by analysis of pond outflow. Secondly, other metabolic products were present in unknown quantities, also the fish in the rearing pond were actively feeding and experiencing disturbances (pond cleanings, etc.). Furthermore, Burrow's work was carried out with Chinook fingerlings whereas our program was carried out with coho pre-smolts.

Burrows believes that ambient ammonia not only leads to gill fungus, but also reduces stamina and growth rates. Exposure for 12 hours per day to ammonia concentrations of 0.10 mg/ℓ and above results in reduced growth rates whereas continuous exposure leads to a reduction in stamina and disease resistance

Burrows, 1964). These effects were noted at pH = 7.8 and water temperature 15°C. Using formula V (Appendix 2) it was found that ammonia levels would have to be 0.15 mg/ℓ for 12 hours per day at Big Qualicum before growth rates would be affected (assuming coho pre-smolts and Chinook fingerlings have similar responses to ammonia).

The diurnal ammonia measurements showed that Big Qualicum coho were exposed to ammonia concentrations of at least 0.15 mg/ℓ for 12 hours per day over the entire test period. In fact the fish were exposed to harmful levels of ammonia 78% of the time. Because the ammonia concentration dropped below 0.15 mg/ℓ for a brief period each night; continuous exposure did not occur.

The intermittent nature of ammonia exposure may be significant. Stamina trials carried out in swim tubes showed a reduced performance of fish continuously exposed to the same level of ammonia. The performance index of fish exposed to ammonia continuously was 16 units whereas the control fish measured 23 units (a 3 unit difference was judged significant) (Burrows, 1964). Perhaps dangerous levels of ammonia in a rearing pond are indicated when daily minimum values are greater than 0.1 mg/ℓ (0.0017 mg/ℓ un-ionized ammonia). When this occurs exposure to harmful levels is continuous and stamina is reduced.

Many general references can be found throughout the literature concerning the harmful effects of ammonia. In most experiments dealing with ammonia toxicity, fish are subjected to high concentrations of ammonia so that the effect under study is easily measured. Although hatchery fish seldom experience such high concentrations, these effects are important in appreciating the sub-lethal responses of fish to ammonia.

According to Brockway (1950), an increase in ammonia lessens the hemoglobins' ability to combine with oxygen or liberate dissolved carbon dioxide. This effect is measurable at an ammonia concentration of only 0.3 mg/ℓ. At ambient ammonia

concentrations of 1.0 mg/ℓ the oxygen content of the blood decreased to about 15% of its normal value. In extreme cases the fish would actually suffocate. In citing these figures Brockway made no reference to temperature or pH.

Lloyd and Orr (1968) have shown that the rate of urine excretion by rainbow trout increases with a rise in the concentration of ambient un-ionized ammonia. It was indicated that exposure to sub-lethal levels of ammonia increase the permeability of the fish to water and that the change in the rate of urine flow is a direct result of an increase in permeability. Perhaps death occurs when the increase in permeability exceeds the maximum sustained rate of urine production (about 12 mℓ/kg/hr).

It was also noted that there was a time lag in the diuretic response. This was attributed to the fact that ambient ammonia does not work directly on the gills but only serves to slow down the rate of ammonia excretion. The ammonia accumulated in the body of the fish and in the gill tissue causes the increase in water permeability.

In this view, any factor which either increases the water permeability (handling, disease, etc.,) or slows down the rate of ammonia detoxification serves to increase the susceptibility of fish to ammonia poisoning. For example, handling causes a diuretic response in fish and, therefore, could be expected to increase their sensitivity to ammonia (Lloyd and Orr, 1968). On the other hand, lower water temperatures may slow down detoxification reactions in the fish. This possibility might explain the fact that the toxicity of un-ionized ammonia increases with decreasing water temperatures.

Fromm and Gillette, (1968) have noted significant changes in nitrogen metabolism of rainbow trout subjected to high levels

of ambient ammonia. Isolated rainbow trout were exposed to NH_4Cl soltuions containing 0, 3, 5 and 8 mg/ℓ of ammonia nitrogen (un-ionized and ionized). It was found that total nitrogen excreted and the nitrogen excreted as ammonia dropped with increasing levels of ambient ammonia. It was further noted that the nitrogen excreted as ammonia decreased at a faster rate. (Fig. 14).

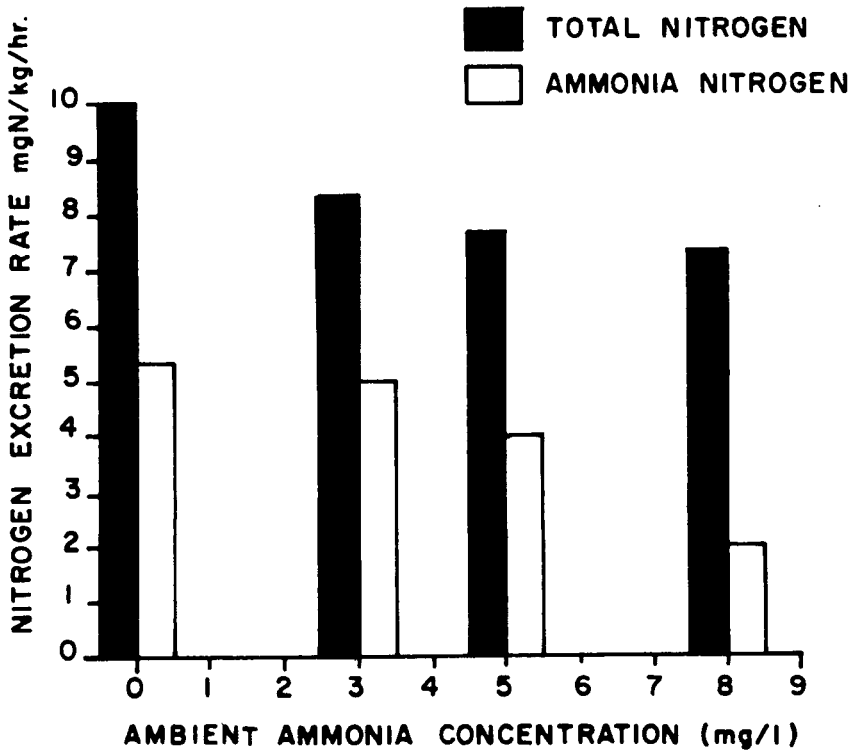

FIGURE 14: THE EFFECT OF AMBIENT AMMONIA ON NITROGEN EXCRETION (FROMM AND GILLETTE, 1968).

Ammonia, being excreted through the gills by passive diffusion is trapped in the fish by high concentrations of ambient ammonia. Apparently the fish respond to this build-up by detoxifying the ammonia. This is accomplished by synthesizing amino acids

and possibly urea, thereby decreasing the free ammonia concentration of the blood. In the manufacture of various amino acids, ∝Keto acids (Pyruvate, ∝Ketoglutarate and oxaloacetate) are diverted from the Citric acid cycle to the transamination reaction. This results in the stimulation of the glycolytic pathway with the resulting build-up of lactic acid. It seems that the fish must accept this condition rather than allow the blood ammonia concentration to increase.

1.5 Summary

(a) Over the period of study ammonia production followed a daily rhythm; concentrations of ammonia reached a peak in the afternoon (1300 to 1900) and fell to a minimum in the early morning (0300 to 0800). The mean daily ammonia nitrogen production was related to the daily ammonia nitrogen ration. It was found that on the average, 48% (range 28% to 80%) of the protein nitrogen ration was accounted for as ammonia nitrogen excretion.

(b) Peak and mean daily ammonia production increased with temperature. Since ammonia production is directly related to food intake, these relationships were assumed to hold because food was available in surplus quantities.

(c) Diurnal ammonia information provides a realistic indicator of ammonia presence in the rearing pond. Using criteria developed for Chinook fingerlings it was concluded that ammonia concentrations were below those considered to cause gill damage or reductions in stamina. However, ammonia concentrations were consistently above the levels considered to affect growth rates.

(d) Disturbance in a heavily loaded pond was observed to produce high rates of ammonia excretion over several days. During this period the fish were continuously exposed to levels of ammonia considered capable of affecting both growth rates and stamina. It was also noted that on the day of the disturbance the ammonia nitrogen excreted accounted for 80% of the daily protein nitrogen ration.

(e) It was hypothesized that ammonia excretion is stimulated during "High" load rate conditions. Calculations showed that ammonia production during the "High" load rate period (7.5 pounds per gpm) exceeded the expected value by nearly 30%.

2. UREA MONITORING PROGRAM

2.1 Introduction

Ammonia and urea are two major nitrogen excretory products of fish. It is generally accepted that ammonia is the dominant nitrogen waste, comprising from 60% to 90% of the nitrogen excreted (Forster and Goldstein, 1968). However, it has been reported that under specific environmental conditions urea can be the dominant nitrogen waste (Burrows, 1964).

The possibility that urea excretion represents an alternate end product of protein catabolism and that this shift can be induced environmentally has important implications to salmon hatchery operation. This is due to the fact that urea is non-toxic at concentrations encountered in rearing ponds, whereas ammonia is harmful at extremely low sub-lethal concentrations. Understanding the influence of environmental factors on nitrogen metabolism might aid in keeping rearing pond ammonia concentrations to a minimum.

In the present study diurnal urea and ammonia excretion of hatchery held Coho Salmon was measured over a variety of environmental conditions. It was hoped that patterns of urea production could be correlated with particular sets of conditions encountered during the study period.

2.2 Materials and Method

The output from a 50 ft. Burrows circulating rearing pond was analyzed regularly over the test period for ammonia and urea. The pond had been loaded with Coho fry in May, 1972. By the time the present work was undertaken the fish had attained an average weight of 2.8 gms.

Water samples were collected from the rearing pond output well using an automatic sampler (Sigma motor type). Samples required for ammonia analysis were fixed with mercuric chloride solution at the time of collection. Samples required for urea analysis were left un-fixed. Analysis was carried out the following day.

2.2.1 Urea Analysis. Ammonia determinations were made using the direct Nesslerization procedure. Urea was measured by the Urease method. The enzyme urease, catalyzes the hydrolysis of urea to ammonia and carbon dioxide. Measurement of ammonia nitrogen before and after the introduction of urease gives a measure of the amount of urea nitrogen present in solution. The details of the test are given below:

1. Measure the initial ammonia nitrogen concentration, Ni, of the sample by direct Nesslerization.
2. Transfer 150 mℓ of sample into an erlenmeyer flask.
3. Add 2 mℓ of Tris buffer solution (pH = 8).
4. Add 2 mℓ of urease solution

5. Cover the flask and incubate at 46°C for 4 hours.

6. Filter the solution and measure the ammonia nitrogen present by direct Nesslerization, N_2.

7. The value, $N_2 - N_1$ is the amount of urea nitrogen present.

The urease solution was prepared by crushing 100 mg. of urease tablets in a beaker and slowly adding 50 mℓ of distilled water. Tris buffer (Tris hydroxymethy amino methane chloride) was used because it neither interacted with urease nor interfered with the Nesslers test. Table 4 gives the make-up of Tris buffer at various pH's (Handbook of Biochemistry).

TABLE 4: Tris buffer Composition

50 mℓ. 0.1 M TRIS (HYDROXYMETHYL) AMINO METHANE,
X mℓ 0.1M HCl DILUTED TO 100 mℓ.

X	pH	X	pH
46.6	7.00	29.2	8.00
45.7	7.10	26.2	8.10
44.7	7.20	22.9	8.20
43.4	7.30	19.9	8.30
42.0	7.40	17.2	8.40
40.3	7.50	14.7	8.50
38.5	7.60	12.4	8.60
36.6	7.70	10.3	8.70
34.5	7.80	8.5	8.80
32.0	7.90	7.0	8.90
		5.7	9.00

Apparently jack bean urease shows a sharp optimum at pH 8 in Tris sulfate buffer (Verner, 1960). However, it has been reported that the reaction reaches an optimum velocity at pH = 9 at low substrate concentrations (West and Todd, 1957). Experimentation at pH's greater than 8 might lead to shorter incubation times.

Urea controls were run with every batch of tests. Over the July 28 to October 19 period a total of 34 controls were run. Percentage errors were calculated from:

$$\frac{X - S}{S} \times 100\%.$$

where X = value measured.

S = value of control.

A frequency histogram has been plotted for the percentage errors of the 34 controls (Fig. 15).

A small number of controls showed errors of approximately -50%. It was assumed that deactivation of the enzyme had occurred. Urease was found to be very sensitive to mercury inhibition, therefore all contact with glassware used in the Nessler's test was avoided. To prevent mercury contamination, the glassware was washed in concentrated HNO_3 and used exclusively for the urea test.

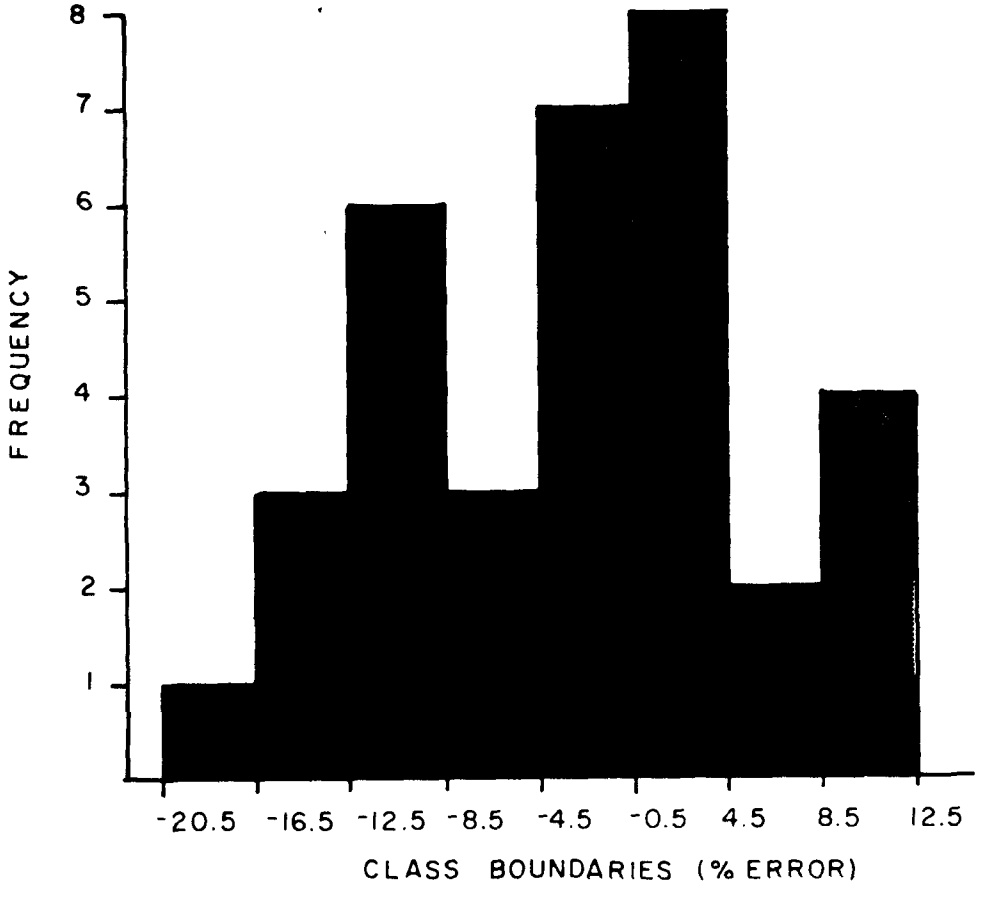

FIGURE 15: PERCENTAGE ERRORS OF 34 CONTROLS. MEAN VALUE -3%, STANDARD DEVIATION 8%.

2.3 Results and Observations

Twelve diurnal samples were taken from July 29 to October 19. Table 6 shows the daily ammonia and urea nitrogen production over the test period. Patterns of urea excretion varied markedly.

Unlike pond ammonia output, which showed a fairly regular maximum and minimum throughout the day, urea output showed no consistent daily rhythm. In an attempt to isolate the effects of environmental changes on urea excretion the data was separted into three groups (Table 5).

TABLE 5. GROUPED UREA DATA

Parameters	Group A.	Group B.	Group C.
Number of Diurnal Samples taken	2	5	5
Time	Mid-Summer (July 29-Aug.5)	Late-Summer Aug. 14-Sept.20)	Early Fall (Oct.3-Oct.20)
Light conditions	Bright	Variable	Bright
Average water temp.	56^{o}F	54^{o}F	52^{o}F
Average pond load rate	2.4 (1.9-2.8)	2.5 (1.6-3.2)	3.8 (3.6-3.9)
Average Fish Size	3.1 gms	6.4 gms	9.6 gms

Table 6. SUMMARY OF UREA PRODUCTION DATA

Date	Period	Nitrogen Production				Protein Nitrogen Ration	Ammonia Excretion Rate mgN/Kg/hr	Urea Excretion Rate mgN/Kg/hr	Temperature °F.
		Mean Ammonia Concentration	Weight of Ammonia	Mean Urea Concentration	Weight of Urea				
July 29-30	0600-0600	0.12 mg/ℓ	262 gms	0.09 mg/ℓ	196 gms	1241 gms	22 mg/Kg/hr	16 mg/Kg/hr	55.5
Aug. 5-6	1600-1400	0.07	140	0.12	240	941	19	33	56.4
Aug. 15-16	1400-1300	0.09	188	n.d.	-	770	21	9	54
Sept.12-13	1030-0630	0.10	229	0.02	36	966	26	-	51.9
Sept.18-19	1100-0800	0.12	242	0.02	36	813	17	3	54.7
Sept.19-20	1300-0800	0.14	104	0.01	17	966	19	3	54.7
Oct. 3-4	1400-0900	0.06	115	0.08	138	610	22	2	52.3
Oct.12-13	1600-1000	0.07	76	0.05	82	407	8	11	50.8
Oct.16-17	1300-1000	0.04	95	0.14	267	687	9	7	51.1
Oct.18-19	1100-0800	0.05	155	0.11	210	915	5	18	50.8
Oct.19-20	1600-1100	0.09	108	0.04	69	407	7	14	51.2
Aug.14-15	1600-0900	0.07		0.03	46	509	12	5	54.1

The diurnal ammonia and urea patterns characteristic of the various groups have been shown in Fig. 16. The graphs have been obtained by averaging over all the sets of data.

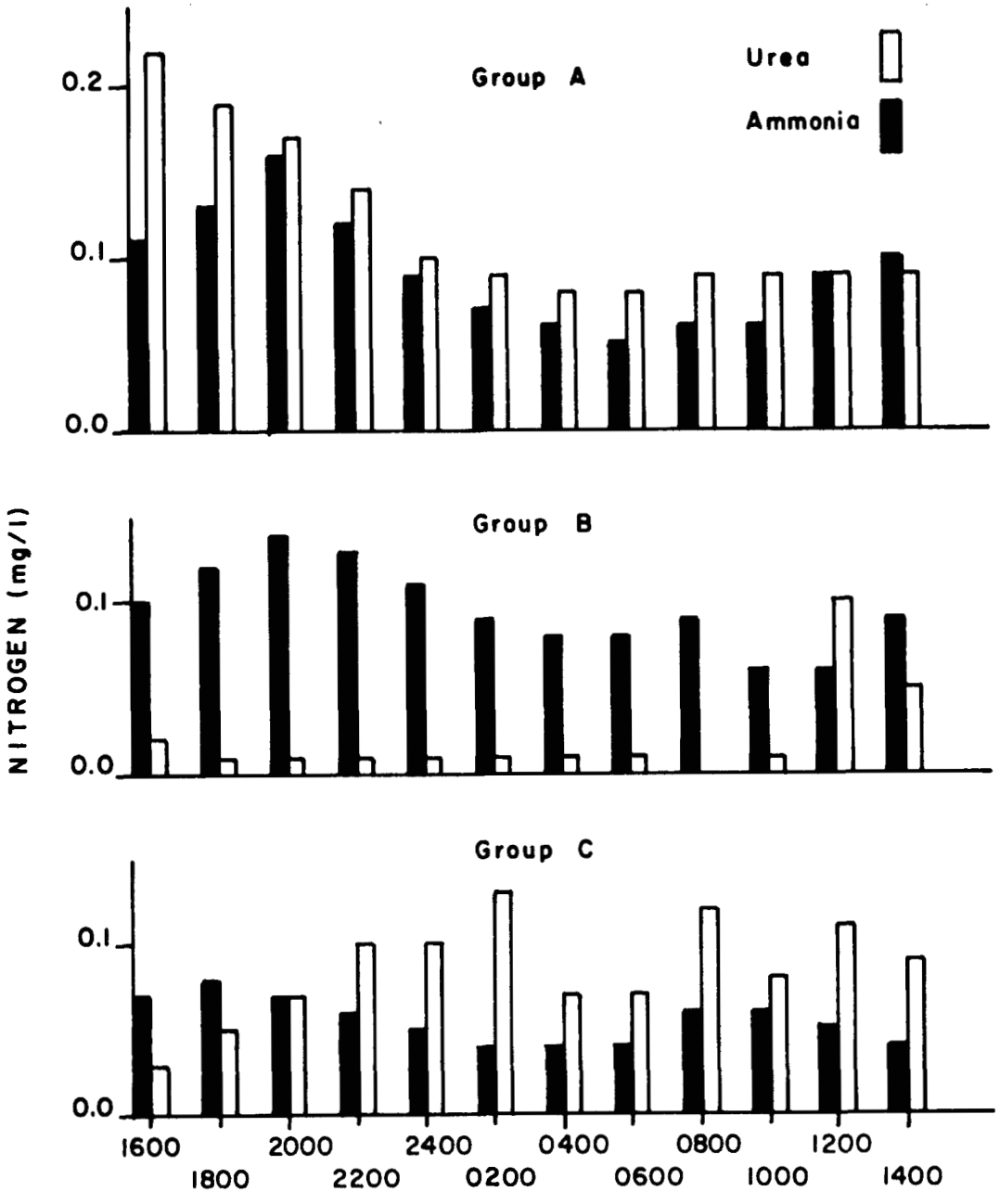

FIGURE 16: GROUP AVERAGE DIURNAL AMMONIA AND UREA PULSES

Ammonia excretion was noted to follow a consistent pattern over the three groups (Fig. 16). Although the magnitude of the pulses varied, ammonia output generally reached a maximum in the afternoon and fell to a minimum in the early morning. Urea excretion showed no overall daily pattern. In group A over 50% of the total nitrogen excretion wastes were in the form of urea. Also, daily variations followed in phase with the ammonia pulse. Group B data showed less than 20% of the total nitrogen waste as urea. Apart from an abrupt peak about 1200, urea excretion was suppressed for most of the day.

Group C data was taken over the most consistent environmental conditions. Sunny days and cold clear nights persisted for most of the October sample period. On October 19 however, an abrupt change to overcast rainy conditions occurred. With this change the average daily water temperature rose 1°F. It is interesting to note that urea excretion dropped from 14 mg/kg/hr on October 18-19 to 5 mg/kg/hr on October 19-20.

Approximately 40% of metabolic nitrogen wastes measured over the test period were excreted in the form of urea. Fig. 17 shows daily average ammonia and urea excretion rates. A low negative correlation was noted between the percentage of nitrogen excreted as urea and water temperature over the August 14-October 20 sample period, (Fig. 18). The July 29 and August 5 sample however, fell completely outside the general pattern.

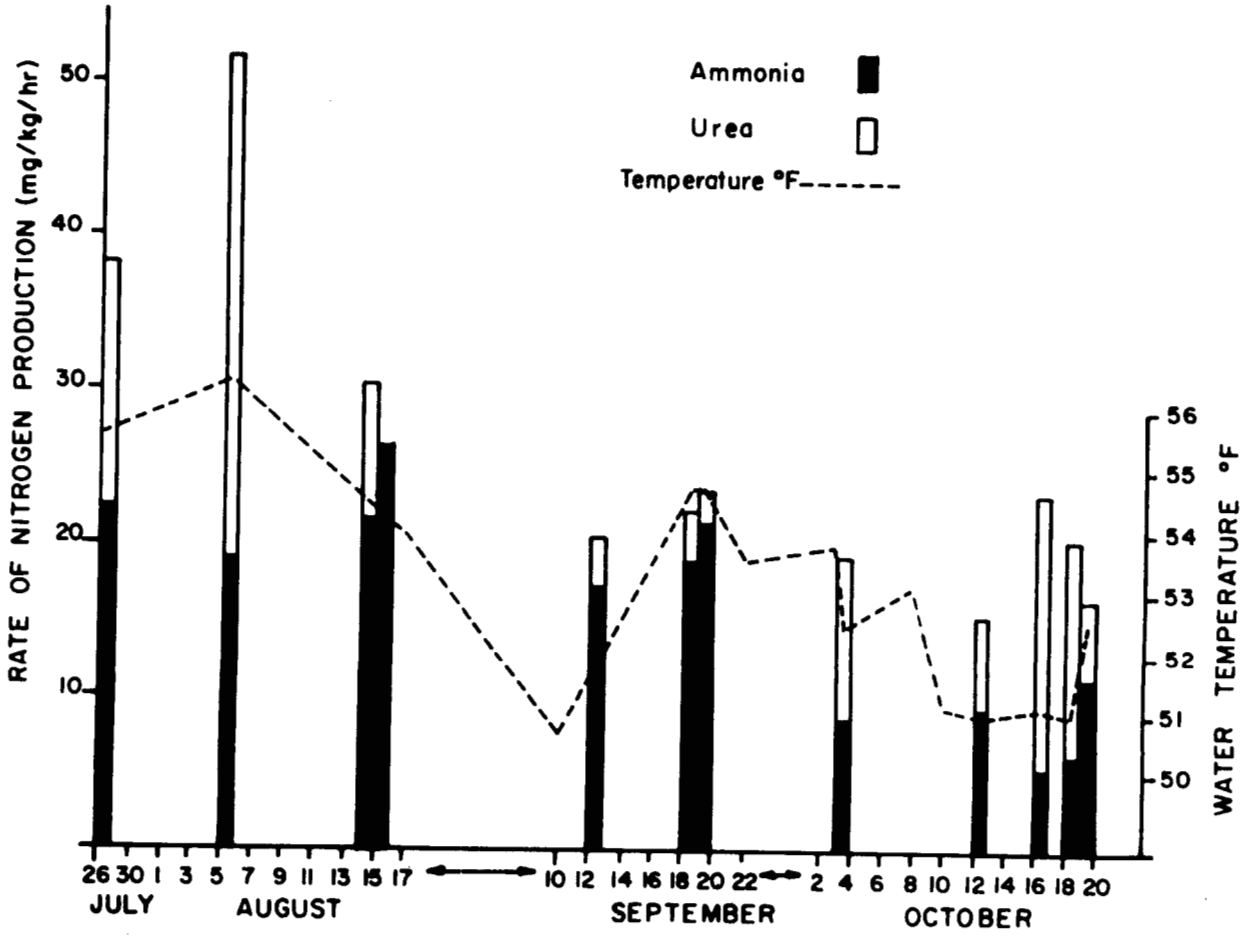

FIGURE 17: AMMONIA AND UREA PRODUCTION OVER THE TEST PERIOD. THE BAR HEIGHT INDICATES THE TOTAL RATE OF NITROGEN EXCRETION (AMMONIA NITROGEN & UREA NITROGEN). THE CLEAR BAR REPRESENTS THE FRACTION EXCRETED AS UREA WHEREAS THE SHADED BAR REPRESENTS NITROGEN EXCRETION AS AMMONIA.

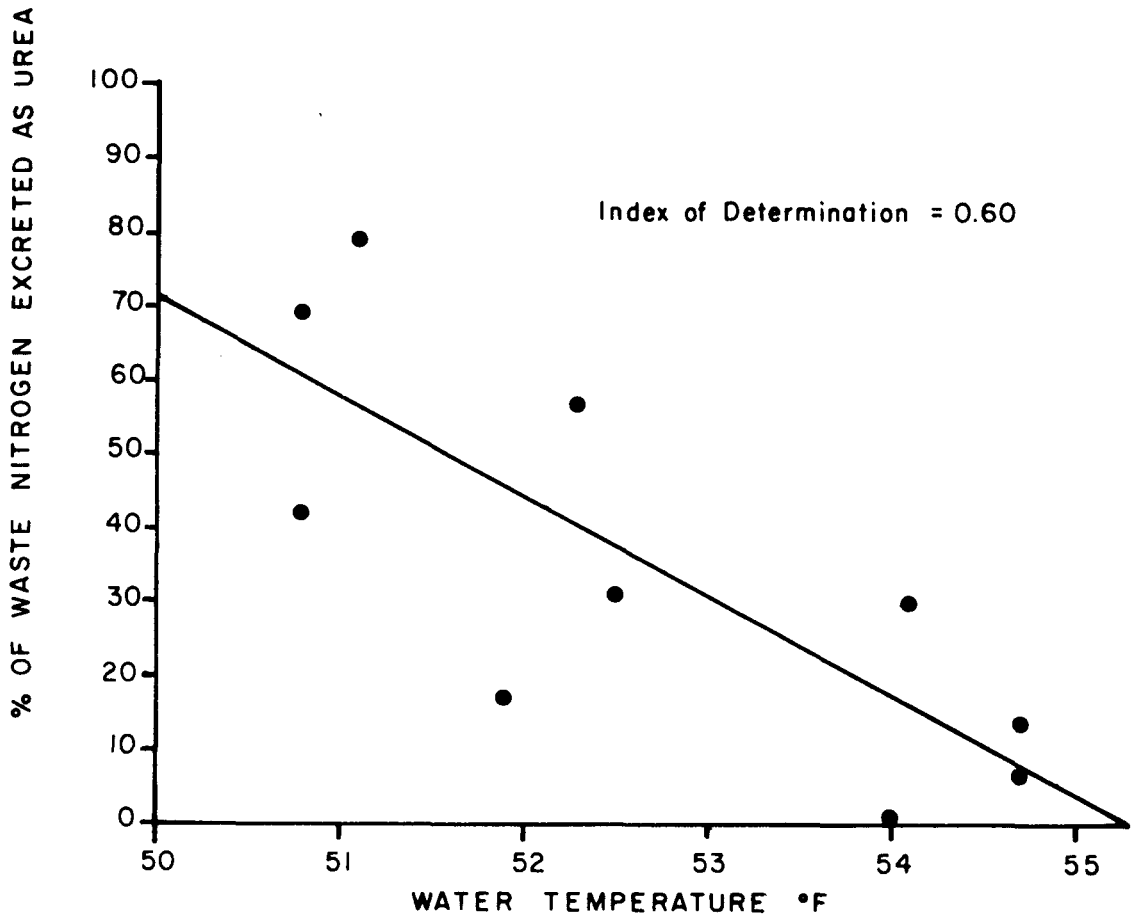

FIGURE 18: PROPORTION OF UREA AS A FUNCTION OF WATER TEMPERATURE. CORRELATION COEFFICIENT = 0.78. (GROUP A DATA HAS NOT BEEN INCLUDED).

Bi-weekly weight samples over the test period showed that 920 pounds of biomass occurred as a result of a 2,403 pound ration. This represents an assimilation of about 10,000 gms. of nitrogen as protein nitrogen. From the small amount of excretion data taken over the test period it was estimated

that between 30% and 45% of the nitrogen ration was excreted as ammonia and urea. Thus, 16% of the nitrogen ration was accounted for in growth and between 30% and 45% in excretion.

On July 29 the study pond was subjected to a typical hatchery disturbance. At 0830 about 500 pounds of fish were removed. Following the transplant, the water level was lowered and the pond cleaned, (1100-1400). At the onset of the disturbance the pond was loaded at about 2.8 pounds per U.S. gpm. with fish averaging 2.8 gm in weight. It is interesting to note the effects of the disturbance on the nitrogen excretion rates of the fish. Fig. 19 shows the suppressed excretion rates following the disturbance. These results are in marked contrast to those obtained following the April 15 disturbance to a heavily loaded pond.

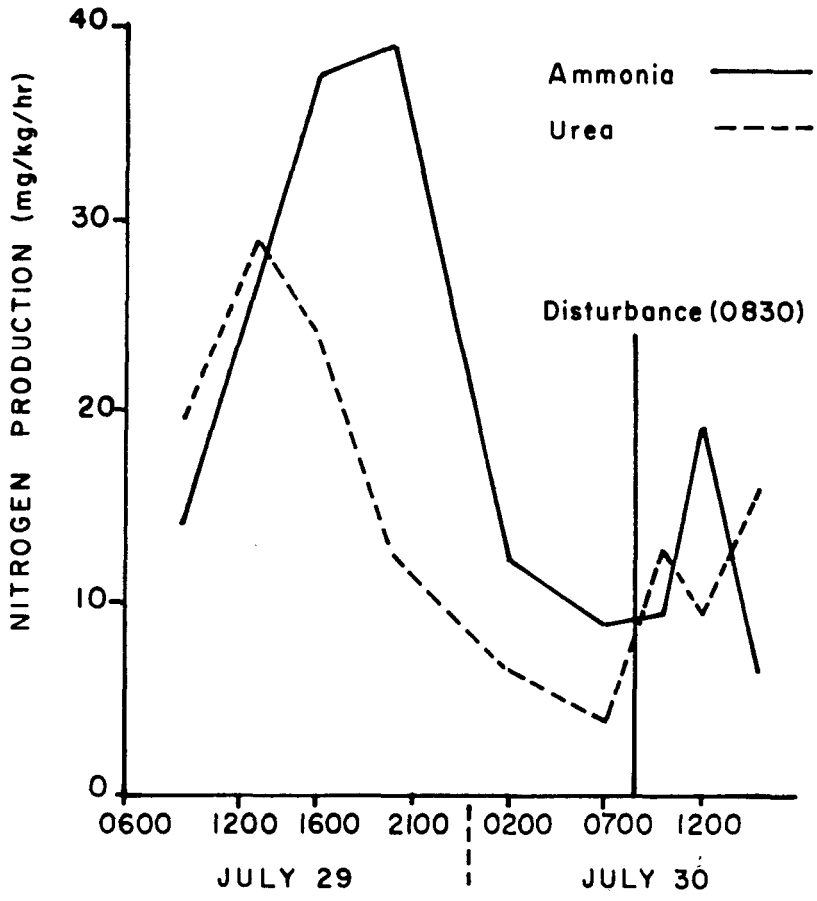

FIGURE 19. NITROGEN EXCRETION FOLLOWING A POND DISTURBANCE.

2.4 Discussion

2.4.1 Urea Excretion under Controlled Conditions.

Little background information is available on the urea excretion of fish under hatchery conditions. However, urea excretion has been measured by Dr. J.R. Brett (pers. Comm.)[2]. In this work, temperature and photoperiod were held constant and the fish were fed a maintenance ration. The diurnal ammonia and urea pattern is shown in Fig. 9. Approximately 13% of the total waste nitrogen was excreted as urea. The low constant rate of urea excretion recorded under maintenace and starvation conditions (Fig. 10) strongly reinforces the view that urea is merely the end product of purine catabolism in fish (Appendix IV).

2.4.2 Urea Excretion under Hatchery Conditiions.

Burrows (1964) claims that under hatchery conditions urea excretion is affected by loading rate and water temperature. Burrows measured the waste output of 4 x 40 foot raceway ponds loaded with Chinook fry. Two ponds were monitored from early June to the end of August. Table 6 shows some of the results of this work. It was concluded that at loading rates above 5 pounds per g.p.m., ammonia became the dominant nitrogen waste product. It was also pointed out that at high metabolic rates (influenced by temperature and fish size), ammonia dominance would be expected at a lower loading rate. This view implies that urea can be a major waste product of protein catabolism in fish.

TABLE 7. PROPORTION OF UREA AT VARIOUS LOADING RATES AND TEMPERATURES (BURROWS, 1964).

Details	Date	Load Rate lb. of fish per gpm	Temperature °F	Approximate % as urea
High inflow pond (250 gpm)	June 8	1.64	46	∿100
	July 20	2.92	52	90
	August 31	4.37	55	60
Low inflow pond (125 gpm)	June 8	3.22	46	95
	July 20	5.60	52	50
	August 31	8.16	55	20

Table 7 summarizes results of the work carried out at Big Qualicum. It was noted that a low negative correlation existed between urea excretion and water temperature over the Groups B and C data. However, inclusion of all data implied that factors other than water temperature and load rate have an important influence on urea excretion.

TABLE 8. PROPORTION OF UREA (BIG QUALICUM RESULTS).

Date	Diurnal Samples	Load Rate lb. per gpm	Temperature °F.	Percent as urea
August (Group A)	2	2.4	56	57
September (Group B)	5	2.5	54	17
October (Group C)	5	3.8	51	56

The environmental factor with the most consistent effect on urea excretion seemed to be the photoperiod. On five out of the twelve sample days the photoperiod was sharply reduced due to overcast rainy conditions.

On two of these days light penetration was further reduced due to water turbidity resulting from river maintenance. Urea accounted for an average of 16% (range 0% to 31%) of the nitrogen excreted on low light days. This was in sharp contrast to urea excretion measured during more intense light conditions. Over these conditions urea accounted for an average of 53% (range 17% to 78%) of the nitrogen excreted.

Environmental conditions were stable during October. Bright sunny days, cool clear nights and falling water temperatures persisted. During this period urea excretion was consistently high. In agreement with Burrows, the diurnal urea pattern was noted to peak during the evening when ammonia excretion was at a minimum (Fig. 16, Graph C). The diurnal nature and magnitude of the urea pulses encountered in the Groups A and C data implies that urea, as well as being the nitrogen waste of purines, canalso be an end product of protein catabolism.

Patterns of nitrogen excretion may be very dependent on environmental conditions. The present work involves Coho with almost unlimited opportunity to feed. During the sample period the average fish size increased from 2.8 gms to 9.9 gms. This was in contrast to the maintenance reation fed to sockeye under controlled conditions.

Burrows found that ammonia excretion dominated in an unfavorable environment. It was suggested that few references exist to urea excretion because of the experimental procedures most often employed to measure excretory products. Usually waste products are accumulated in small quantities of water over long periods of time. This procedure results in conditions similar to those encountered at high loading rates during which ammonia predominates and urea appears insignificant.

The July 30 pond disturbance did not cause increases in nitrogen excretion as did the disturbance of April 15. This could be attributed to the fact that the July disturbance involved a reduction in load density from 12.7 gms. per liter to 7.0 gms. per liter whereas the April disturbance involved a reduction from 28 gms. per liter to 23 gms. per liter. The impact of such disturbances on the fish appears to be related to the initial pond load density.

2.5 Summary

(a) Urea is an important nitrogen excretory product of salmon rearing under hatchery conditions. Over the study period urea excretion averaged 8.8 mgN/kg/hr. - accounting for 36% of the rearing pond nitrogen wastes.

(b) Urea excretion was noted to be variable. On particular days it accounted for between 0% and 78% of the nitrogen excreted. The environmental conditions responsible for stimulating urea excretion could not be clearly defined due to the limited data collected. However, consistently high urea excretion rates were measured during October. This period was characterized by low water temperatures and intense light conditions.

(c) The diurnal urea pulse did not follow as regular a pattern as the daily ammonia pulse. However, the September and October samples showed that urea concentrations reached a maximum during the early morning (0100-0900) when ammonia concentrations were at a minimum.

(d) Disturbance to a rearing pond loaded at 12 gms. per liter did not cause an increase in the nitrogen excretion rates. This was in direct contrast to the high ammonia concentrations following the April disturbance to a pond loaded at a density of 28 gms. per liter.

3. CONCLUSIONS

Ammonia and urea excretion measurements were carried out over a wide range of hatchery conditions. Ammonia excretion ranged from 8 mg/kg/hr for 11.3 gm coho pre-smolts in March to 22 mg/kg/hr for 2.8 gm coho fry in July. Limited urea analysis over the Summer and Fall periods showed more variable results. Urea excretion averaged 9 mg/kg/hr. It was assumed that particular environmental conditions stimulated urea excretion. Although these conditions could not be clearly defined from the data available, it appeared that urea excretion for a given load rate and feed rate was associated with low temperatures and intense light conditions.

Ammonia nitrogen excretion patterns followed a diurnal rhythm. Ammonia output was at a minimum in the early morning and reached a maximum during the afternoon. Urea excretion, on the other hand, was more variable. During August, urea output followed in phase with the diurnal ammonia pulse. However, during the stable environmental conditions of October, urea production peaked in the early morning when ammonia excretion was at a minimum.

During the Spring program it was estimated that the coho pre-smolts excreted nearly 50% of the protein nitrogen ration as ammonia. A theoretical calculation, based on a rough estimate of the growth rate and the assumption that 15% of the growth was protein, showed that about 18% of the nitrogen ration was assimilated as growth. In this way nearly 70%

(68% $\pm$ 5%) of the nitrogen ration was accounted for. Presumably the remainder was excreted as urea or lost directly as waste. Similar calculations over the Summer-Fall period however, showed that the sum of the protein nitrogen assimilated and the ammonia and urea nitrogen excreted accounted for only 54% $\pm$ 8% of the nitrogen ration. Although these calculations involve a high degree of uncertainty they imply greater food waste over the Summer-Fall period.

Diurnal ammonia and pH patterns accurately reflected the daily exposure of fish to un-ionized ammonia. Since ammonia exposure has been related to reductions in growth rates, stamina and disease resistance (Burrows, 1964) regular ammonia measurements would indicate potentially harmful situations. Ammonia concentrations measured over the Spring period were capable of affecting growth rates. However, concentrations encountered during the first two weeks of April might also have caused reductions in stamina. It should be pointed out that these effects were not demonstrated - predictions were based entirely on criteria developed for Chinook fingerlings.

Nitrogen excretion measurements might have more wide ranging implications to the operation of salmon rearing facilities. It has been suggested that values for daily nitrogen excretion may be a more reliable parameter than oxygen consumption in assessing the metabolic effects of stress on fish (Fromm, 1963). For example nitrogen excretion was noted to increase disproportionately during periods of low dissolved oxygen and during forced activity. Also pond load rate was thought to have an effect on the rate of nitrogen excretion. Burrows (1964) suggested that the type of nitrogen waste (ammonia or urea) also reflected the state of the fish. Perhaps other environmentally induced stress states are similarly reflected in the daily patterns of nitrogen excretion.

In order to quantify the effects of environmental change on nitrogen excretion, ammonia and urea measurements would have to be coupled with oxygen consumption data. This would allow association of environmental changes with characteristic changes in the ammonia and urea quotients[4]. Such information would show whether increasing nitrogen production results from a significant increase in protein utilization or whether it is simply the result of increased metabolic rates.

For example, measurements of oxygen consumption, ammonia excretion and urea excretion during the April rearing pond disturbance and during the high load period would have permitted calculations of changes in UQ and AQ. This would have shown whether the increased ammonna production resulted from:

(a) Increased metabolic rates due to excitation, perhaps from crowding - indicated by AQ and UQ remaining constant.

(b) Greater demand being made on protein catabolism - indicated by an increase in AQ + UQ.

(c) Greater demand being made on the pathway of protein catabolism leading to ammonia excretion - indicated by an increase in AQ and a corresponding decrease in UQ.

External factors that cause sudden shifts to protein catabolism would tend to decrease growth rates and increase ambient ammonia concentrations.

(4) $$\text{AQ} = \text{Ammonia Quotient} = \frac{\text{Volume of Ammonia Excreted}}{\text{Volume of Oxygen Consumed}}$$

$$\text{UQ} = \text{Urea Quotient} = \frac{\text{Volume of Urea Excreted}}{\text{Volume of Oxygen Consumed}}$$

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APPENDIX I

Typical proximate analysis of Oregon Moist Pellet (Hoblou, 1963).

Protein	34.8%
Moisture	34.4%
Carbohydrate (by difference)	16.5%
Fat	7.5%
Ash	6.8%
Digestible Calories	1008 Kcal per lb.

APPENDIX II

The relationship between ionized ammonia, NH_4^+ and un-ionized ammonia NH_3 is a function of both pH and temperature.

(a) pH Dependence: The ionization constant, K, for aqueous ammonia is defined by:

$$(i) \quad K = \frac{[NH_4^+]\ [OH^-]}{[NH_3]}$$

The dissociation constant of water Kw is defined by:

$$(ii) \quad Kw = [H^+]\ [OH^-]$$

At a given pH condition in the pond

$$[H^+] = 10^{-pH} \text{ and } [OH^-] = Kw10^{pH}.$$

Substituting the expression for $[OH^-]$ into (i) gives

$$(iii) \quad K = \frac{[NH_4^+]\ Kw10^{pH}}{[NH_3]}$$

Since the Nesslers test measures the total ammonia, "A", (un-ionized + ionized ammonia)

$$(iv) \quad A = [NH_4^+] + [NH_3]$$

Combining equation (iii) and (iv) and rearranging allows expression of the ratio of un-ionized ammonia NH_3 to total ammonia A, as a function of pH:

$$(v) \quad \frac{[NH_3]}{A} = \frac{1}{1 + \frac{K}{Kw10^{pH}}}$$

(b) Temperature Dependence: As temperature increases, K and Kw increase. The net effect is that the percentage of un-ionized ammonia in a given aqueous ammonia solution increases with temperature.

K and Kw values at different temperatures have been compiled from the Hand Book of Chemistry and Physics (Table 9). Table 10 shows percent un-ionized ammonia as a function of pH and water temperature.

TABLE 9. K AND Kw AT DIFFERENT TEMPERATURES.

Temperature	K	-log 10 Kw
5°C	1.479 x 10-5	14.7338
10°C	1.570 x 10-5	14.5346
15°C	1.652 x 10-5	14.3463
20°C	1.710 x 10-5	14.1669

TABLE 10: PERCENT UN-IONIZED AMMONIA VS. pH AT 10°C, 15°C, AND 20°C.

Percent un-ionized ammonia.

pH	Temperature = 10°C	Temperature = 15°C	Temperature = 20°C
6.5	0.059%	0.086%	0.126%
6.6	0.074	0.108	0.158
6.7	0.093	0.137	0.200
6.8	0.117	0.172	0.251
6.9	0.148	0.216	0.315
7.0	0.186	0.272	0.397
7.1	0.234	0.342	0.499
7.2	0.294	0.430	0.627
7.3	0.370	0.541	0.788
7.4	0.465	0.681	0.990
7.5	0.585	0.855	1.243
7.6	0.735	1.074	1.560
7.7	0.924	1.349	1.956
7.8	1.160	1.692	2.450
7.9	1.350	2.121	3.065
8.0	1.826	2.655	3.829
8.1	2.288	3.320	4.773
8.2	2.863	4.144	5.934
8.3	3.578	5.162	7.358
8.4	4.463	6.413	9.091
8.5	5.555	7.942	11.182
9.0	22.598	21.433	28.474
9.5	37.037	46.318	55.72
10.0	65.032	73.175	79.92
11.0	94.897	96.464	97.50
12.0	99.465		

APPENDIX III

Apparently the molecular condition of ammonia in water is uncertain. Some authors report aqueous ammonia as ammonium hydroxide (NH_4OH) while other authors refer to it as ammonia hydrate ($NH_3 \cdot H_2O$) or simply $NH_3(aq)$. According to Cotton and Wilkinson (1962) use of the term ammonium hydroxide should be discouraged because it is doubtful whether un-dissociated NH_4OH exists. In this view solutions of ammonia in water are best described as $NH_3(aq)$ with the equilibrium written as:

$$NH_3(aq) + H_2O \rightleftharpoons NH_4^+ + OH^-.$$

Remy (1960) makes a similar distinction between aqueous ammonia and ammonium hydroxide. Remy claims that the compound ammonia hydrate ($NH_3 \cdot H_2O$) probably exists in equilibrium with NH_4^+ and NH_3 but that NH_4OH is definitely not present in the un-dissociated form. Remy writes the equilibrium as:

$$NH_3 + H_2O \rightleftharpoons NH_3 \cdot H_2O \rightleftharpoons NH_4^+ + OH^-$$

$$K = \frac{[NH_4^+]\,[OH^-]}{[NH_3]}$$

The point is made that "K" may be spoken of as the electrolytic dissociation constant of ammonia hydrate but that it is logically incorrect to refer to it as the dissociation constant of ammonium hydroxide.

To avoid confusion, water solutions of ammonia are referred to as aqueous ammonia (NH_3aq) in this report. The symbol NH_3 represents un-ionized ammonia while NH_4^+ represents the ionized form.

APPENDIX IV

Ammonia is the immediate nitrogen waste product of amino acid catabolism. Mammals escape the harmful effects of free ammonia by synthesizing urea. The net reaction is as follows:

$$3ATP + 2NH_3 + CO_2 \rightleftharpoons O = C(NH_2)_2 + H_2O + 3ADP$$

Ammonia Urea

This reaction is carried out via the ornithine cycle and, as indicated above, requires considerable energy (ATP).

Fish escape the harmful effects of ammonia by continuously excreting it through their gills. Little information is available concerning urea formation in fish. Apparently fish cannot detoxify ammonia by synthesizing urea via the ornithine cycle. According to Forster and Goldstein (1965) three critical ornithine cycle enzymes are lacking in fish.

The known sources of urea in fish are as follows:

1) Dietary Arginine.

Arginine is an essential amino acid. The enzyme "arginase" is present in the liver of fish so the following reaction is possible:

Arginine $\xrightarrow{\text{Arginase}}$ urea + ornithine.

Since arginine is an essential amino acid it is unlikely that dietary arginine makes a significant contribution to urea formation in fish.

2) Purine Catabolism.

Uric acid is the immediate waste product of purine catabolism. Since the enzymes required to convert uric acid to urea are present in fish, urea is considered to be the ultimate waste product of purine catabolism. The net reaction is as follows:

Purine $\longrightarrow$ Uric $\longrightarrow$ Allantoin $\longrightarrow$ Allantoic $\longrightarrow$ Urea + Glyoxylic Acid

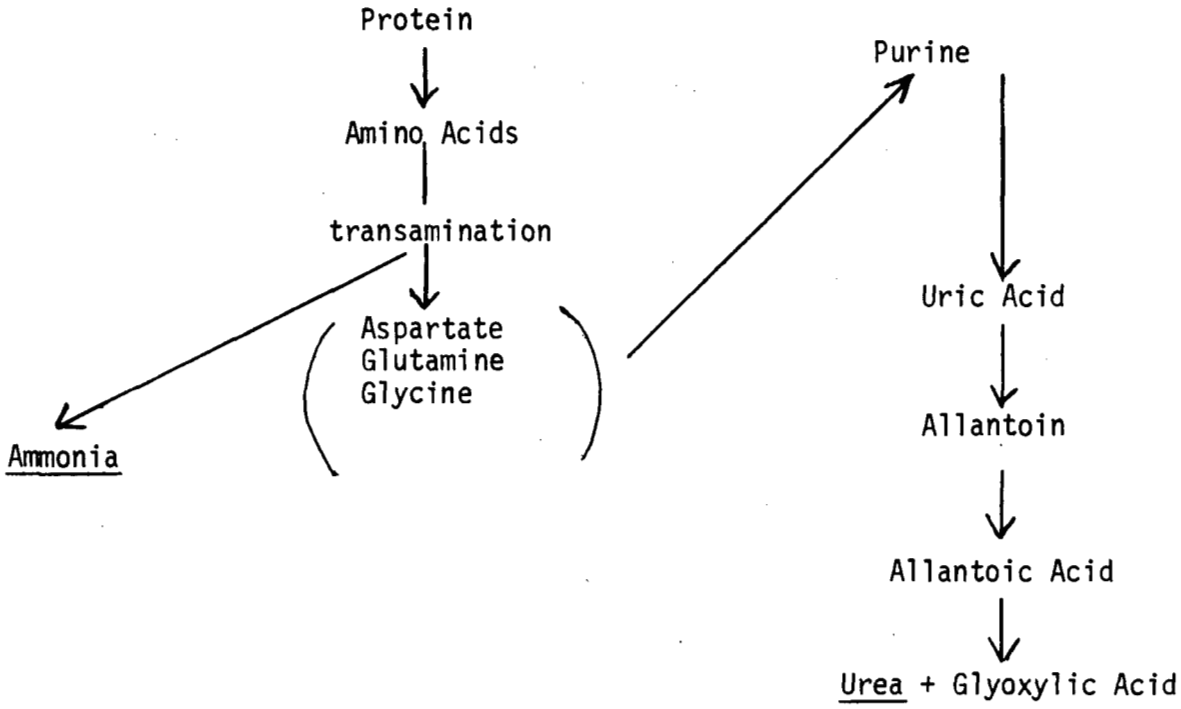
Keeping in mind that uric acid is the ultimate waste product of purine catabolism in man it is interesting to compare the uric acid production of humans to the urea production of fish. (Table 11).

TABLE 11. A COMPARISON BETWEEN URIC ACID EXCRETION OF MAN AND UREA EXCRETION OF FISH.

Species	Diet	Percent of total Nitrogen excreted as uric acid.	Reference
Man	High Protein	1.3%	West and Todd (1957)
"	No Purine	0.5%	" " "
"	High Purine	∿10%	" " "
"	Starvation	1.9%	" " "
Species	**Diet**	**Percent of N. excreted as urea**	**Reference**
Sockeye	High Protein(OMP)	13%	FRB-unpublished
Sockeye	Starvation	26%	" "
Fish (unspecified)	unspecified	∿20%	Goldstein & Forster (1965)
Sculpin	High Protein	20%	Wood (1958)
Blue Sea Perch	" "	38%	" "
Chinook	High Protein(OMP)	0 to ∿90%	Burrows (1964)
Coho	" " "	40%	Big Qualicum Results

The urea excretion of fish accounts for a significantly greater percentage of waste nitrogen than the uric acid excretion of man. Such high levels of urea excretion lead to speculation that urea represents more than just a waste product of purine catabolism.

Goldstein and Forster (1964) suggest that urea (like ammonia) may be a waste product of amino acid catabolism. The hypothetical pathway is as follows:



The purine ring is synthesized from the amino acids glutamine, aspartate and glycine. Degradation leads to the production of urea. In this view urea excretion represents an alternate pathway for the disposal of waste nitrogen.