SURVIVAL, SIZE, AND EMERGENCE OF PINK SALMON, *ONCORHYNCHUS GORBUSCHA*, ALEVINS AFTER SHORT- AND LONG-TERM EXPOSURES TO AMMONIA

STANLEY D. RICE AND JACK E. BAILEY[1]

ABSTRACT

Eggs and alevins of pink salmon, *Oncorhynchus gorbuscha*, were exposed to ammonia in a series of static and flow-through experiments to determine what levels of ammonia would decrease survival. Short-term acute toxicity tests (96 hours) were conducted at several stages in development to determine which of the early life stages were most sensitive to ammonia. Long-term tests (up to 61 days) with lower ammonia concentrations were conducted to determine effects on survival and size of fry at emergence. The possibility of ammonia stimulating emergence of immature fry was tested at various stages of development.

Pink salmon alevins were most sensitive at the completion of yolk absorption, when the 96-hour median tolerance limit was 83 parts per billion of un-ionized ammonia. Concentrations as low as 1.2 parts per billion reduced fry length in the 61-day exposures. Only levels above 10 parts per billion of ammonia stimulated early emergence of immature fry.

The concentrations of ammonia causing any of the deleterious effects observed are greater than concentrations observed in the hatchery or the natural environment.

Ammonia is a natural waste product of protein catabolism and can be toxic to aquatic organisms under certain conditions. Ammonia exists in the water in two forms, un-ionized NH_3 and ionized NH_4^+; the NH_3 is much more toxic than NH_4^+ (European Inland Fisheries Advisory Commission 1970). The percentage of ammonia in the more toxic form, NH_3, is influenced primarily by pH of the water and by factors that influence pH, e.g., temperature, carbon dioxide, and bicarbonate alkalinity (European Inland Fisheries Advisory Commission 1970).

Much information is available on the toxicity of ammonia to fishes, including juvenile and adult salmonids; but it is surprising that little information exists on the toxicity of ammonia to fertilized eggs and larvae of teleosts, especially because these life stages are often assumed to be relatively sensitive. In acute toxicity studies, trout eggs and alevins[2] are much more tolerant to ammonia than fry (Penaz 1965; Rice and Stokes 1975). Long-term studies of larval and juvenile forms exposed to ammonia are virtually nonexistent; but in one long-term study Burkhalter and Kaya (1977) continuously exposed rainbow trout, *Salmo gairdneri*, eggs and alevins to ammonia until the end of yolk absorption (about 67 d). They found retardation of growth at the lowest concentrations tested and other adverse effects at higher concentrations.

Toxicity of NH_3 to the early life stages of salmon in the northern latitudes would not be a problem because low temperature and pH cause most of the ammonia (99+%) in the water to be in the less toxic form, NH_4^+. However, salmon eggs and alevins in subarctic latitudes have a long developmental life history in an intragravel stream environment (up to 8 mo) where intragravel waterflow can be reduced for several months during the winter because of low temperatures. The low waterflow may not be sufficient to prevent a buildup of excreted ammonia in the water layer immediately adjacent to the developing egg or alevin. Ammonia levels may rise during these periods of low flow to concentrations that are deleterious to survival, health, and/or growth of the developing salmon.

Our study had three specific objectives: 1) to determine the sensitivities (judged by survival) of early life stages of pink salmon, *Oncorhynchus*

[1]Northwest and Alaska Fisheries Center Auke Bay Laboratory, National Marine Fisheries Service, NOAA, P.O. Box 155, Auke Bay, AK 99821.

[2]After hatching, alevins (salmon larvae) reside in the spawning gravels for several months while consuming their large yolks. Salmon alevins that have no externally visible yolk ("buttoned up") become fry when they inflate their swim bladder and become free-swimming; this occurs immediately after volitional emergence from natural redds and gravel incubators.

Manuscript accepted January 1980.
FISHERY BULLETIN: VOL. 78, NO. 3, 1980.

gorbuscha, to short-term, high concentrations of NH_3; 2) to determine the size of emergent fry after long-term exposure of alevins to low concentrations of NH_3; and 3) to determine whether early emergence of immature salmon fry can be caused by short-term sublethal concentrations of NH_3. The short-term tests with high concentrations of NH_3 identified the more sensitive life stages and provided information on concentrations that were lethal. The sublethal tests identified NH_3 concentrations that caused decreases in the size of emergent fry or caused early emergence of immature fry. Identification of factors that cause smaller fry at emergence is important because smaller fry are less capable of surviving in the environment. Laboratory and field studies by Bams (1967) and Parker (1971) have shown that smaller salmon fry have less swimming endurance and are selectively preyed upon by larger predators.

Our ultimate objective was to compare the concentrations of NH_3 that are harmful to pink salmon in the laboratory with concentrations of NH_3 that are harmful in hatchery incubators (Bailey et al. 1980) and in natural spawning redds (Rice and Bailey 1980). To compare the studies of Bailey et al. and Rice and Bailey with our study, our tests were conducted with pink salmon eggs, alevins, and fry exposed to NH_3 at temperatures <4.8° C and pH's <6.5—conditions that are typical for freshwater streams in boggy rain forests of the colder northern latitudes.

MATERIALS AND METHODS

The pink salmon eggs were fertilized in September at Lovers Cove Creek, southern Baranof Island, southeastern Alaska, and incubated to the eyed stage in Heath[3] incubators at Auke Bay, near Juneau, Alaska. Some eyed eggs were taken from the Heath incubators and placed in upwelling incubator cups or in 15.2 cm diameter pipe incubators (Rice and Moles[4]) for long-term exposures or emergence stimulation tests. The rest of the eggs were left in the Heath incubators for short-term bioassays.

We measured concentrations of total ammonia (NH_3 + NH_4^+) by an automated method that measured the intensity of indophenol blue formed after the reaction of ammonia with alkaline phenol hypochlorite (U.S. Environmental Protection Agency 1974). Total ammonia concentrations were analyzed the same day water samples were taken. The concentrations reported in this study are for the toxic, un-ionized NH_3. Total ammonia was measured and the concentration of NH_3 determined by using the temperature-pH correction tables of Emerson et al. (1975).

Our experimental approach involved three types of experiments: 1) to determine the sensitivity (survival) of each early life stage to NH_3, we exposed eyed eggs, alevins, and fry to short-term, high concentrations of NH_3 (>50 ppb); 2) to determine the effect of long-term exposures of NH_3 on size of fry at emergence (end of yolk absorption), we exposed alevins at different stages of development to low concentrations of NH_3 (<3 ppb) for up to 61 d; and 3) to determine whether NH_3 would cause emergence of immature fry, we exposed alevins to high concentrations of NH_3 (30-150 ppb) for 24 h and counted voluntary out-migrants from the incubators.

The senstivities of different life stages to short-term, high concentrations of NH_3 were tested with 96-h bioassays conducted according to the standard procedures of Doudoroff et al. (1951). Eggs, alevins, and fry were exposed to static solutions of ammonium sulfate in freshwater at pH of 6.3-6.5 and 3.7°-4.8° C. Twenty-five animals were placed in each 18 l test container; resulting ratios of tissue to test solution were <0.3 g/l. The test solutions were aerated and the tests were conducted in the dark. Median tolerance limits (TLm's) and associated 95% confidence levels were calculated by a computerized probit analysis program based on the method discussed by Finney (1971).

To test the effect of long-term exposures to NH_3 on size of fry at emergence, ammonium sulfate was introduced into the water which flowed through incubator cups containing the developing alevins. Twenty-five eyed eggs were placed in each upwelling incubator cup (Bailey[5]), and exposures to NH_3 began at selected times after hatching. The NH_3 was introduced by dripping small quantities of concentrated ammonium sulfate solutions into

[3]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

[4]Rice, S. D., and D. A. Moles. 1977. Apparatus for incubating salmonid eggs and alevins in a variety of controlled environments for laboratory studies. Unpubl. manuscr. Northwest and Alaska Fisheries Center Auke Bay Laboratory, NMFS, NOAA, Auke Bay, AK 99821.

[5]Bailey, J. E. 1964. Incubation of pink salmon eggs in a simulated intertidal environment. *In* Proceedings 15th Annual Northwest Fish Culture Conference, p. 79-89. Oregon Agricultural Experiment Station, Corvallis, OR 97330.

the incoming water line to each cup. Flow rates of both water and ammonium sulfate were kept constant by using supplies with a constant head. We measured flow rates daily and concentrations of total ammonia in each cup twice each week.

In the long-term tests, three groups of alevins (A, B, and C) were exposed to ammonium sulfate solutions for three different lengths of time. Group A was exposed for the 21 d preceding the completion of yolk absorption (time of normal emergence and migration). Group B was exposed for 40 d, with the exposure ending 21 d before yolk absorption. Group C was exposed for 61 d before yolk absorption. In each group, subgroups of alevins were exposed to concentrations of NH_3 ranging from 0 (control) to 4 ppb.

At the end of the long-term exposures when control fry had absorbed all visible yolk, 50 fry from each NH_3 exposure were sampled and preserved in 5% Formalin. Size was determined after 6 wk when tissue hydration adjustments were stable. After blotting the fry with a damp paper towel, fork lengths were measured to the nearest millimeter and weights to the nearest milligram. Mean K_D developmental indices (Bams 1970) were 1.99 for the controls and 1.96-2.07 for the groups exposed to NH_3; therefore, fry were at similar developmental stages and the size of fry from the various groups could be compared directly with the size of controls. Data are reported as means ±95% confidence intervals.

In another experiment, the possible stimulation of early emergence of immature fry was tested by exposing alevins in gravel incubators to a single exposure of NH_3 for 24 h at various times during development. Concentrated ammonium sulfate solutions were pumped into the intake lines of small experimental gravel incubators (Rice and Moles footnote 4) at rates sufficient to produce desired concentrations of NH_3. Alevins or fry were trapped if they voluntarily emerged during the exposure. The numbers of fry and stage of development were noted daily. Pink salmon were judged to have emerged early when they emerged before control fry and were judged to have emerged prematurely if yolk sacs were visible externally. Pipe incubators with a volume of 5 l and a water flow of 450 ml/min were seeded in November with 300 eyed eggs each. Each week after hatching, several incubators were exposed to four different concentrations of NH_3 for 24 h. Each incubator received only one 24-h treatment and different sets of incubators were used for the NH_3 exposures that were performed every week during the 2 mo prior to emergence of controls. All NH_3 concentrations were measured analytically by the method previously described.

SENSITIVITY OF DIFFERENT LIFE STAGES TO AMMONIA

Late alevins near emergence were the most sensitive of all life stages tested to short-term, acute concentrations of NH_3 (Figure 1). Eyed eggs exposed for 96 h to toxicants in December were not harmed by concentrations >1,500 ppb and appeared to hatch normally. Late alevins (tested 13-17 March) had the lowest 96-h TLm measured, 83 ppb of NH_3. The fry (tested on 10-14 April) had been feeding for 2 wk and appeared to have slightly greater tolerance to NH_3 than the alevins tested just prior to emergence, but the differences between the tests were not statistically significant. Greater sensitivity just prior to emergence is consistent with similar observations of trout eggs and alevins exposed to ammonia (Penaz 1965; Rice and Stokes 1975). Studies with other toxicants have identified eggs to be much more tolerant than alevins (Wurtz-Arlet 1959; Garrison 1968; Rice et al. 1975).

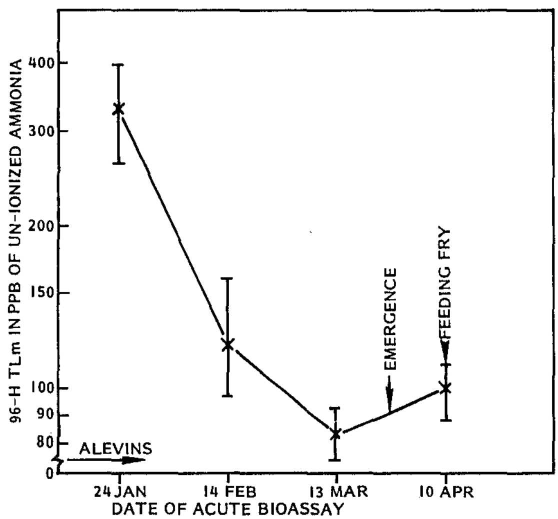

FIGURE 1.—96-h median tolerance limits (TLm's) and ±95% confidence limits of pink salmon alevins and fry exposed during short-term, acute experiments to un-ionized ammonia (NH_3). Eggs were also tested, but no mortalities were observed at the highest concentration (1,500 ppb NH_3).

EFFECT OF LONG-TERM AMMONIA EXPOSURES ON SIZE OF FRY AT EMERGENCE

Lengthy exposure of alevins to NH_3 resulted in fry that were smaller than control fry at emergence (Figure 2). Although the three test groups of alevins (groups A, B, and C) were exposed to NH_3 for different time periods (21, 40, and 61 d), they were all sampled when the control groups had completed yolk absorption. The highest exposure concentration of NH_3 (4 ppb) caused significant decreases in weight ($P<0.05$) of exposed fry in all three exposure groups. At exposure concentrations <4 ppb, the groups held for 40 d and 61 d (B and C) were similar in response: both were significantly smaller in length and weight after exposure to 2.4 ppb NH_3; after exposure to 1.2 ppb there was no significant difference. Effects were consistently more adverse for group C, the group receiving the longest exposures. The statistically significant decrease in weight ($P<0.05$) of one observation of group A exposed to 0.2 ppb of NH_3 appeared to be an aberrant observation.

EFFECT OF AMMONIA ON EARLY EMERGENCE

Short-term (24 h) exposures to low concentrations of NH_3 (<25 ppb) did not stimulate early emergence during or immediately after the exposures; emergence patterns were the same as those of unexposed fry. At higher concentrations (30-50 ppb) of NH_3, early emergence of the alevins (up to 11%) occurred within 24 h of exposures, but little residual effect was observed later when 50% emerged at approximately the same date as unexposed alevins (Figure 3). Some early emergence of the alevins (up to 12%) occurred at high concentrations of 100-150 ppb NH_3, but massive early emergence was not observed even though these concentrations were above the 96-h TLm's for alevins or fry during the period 15 February to 10 April (Figure 1). Although the high concentrations were probably stressful, mortalities never exceeded 4%. In all cases, when early emergence was stimulated, it occurred within 24 h of the beginning of exposure. In response to the acute exposures to NH_3, the alevins that emerged early had visible amounts of yolk, indicating they were not ready for normal emergence. The alevins that did not emerge during or immediately after NH_3 exposures stayed in the incubators almost as long as the unexposed alevins and emerged without any visible yolk.

IMPLICATIONS OF AMMONIA EXPOSURE STUDIES

Ammonia exposures resulting in immature or small fry would be detrimental to the survival of pink salmon fry because these small fish are easily preyed upon (Bams 1967; Parker 1971). We did observe some early emergence of immature fry during or immediately after short-term exposures to NH_3, but only at the high concentrations that aproached highly toxic levels. These high concentrations are not likely to be encountered in natural or hatchery environments, but if they were encountered, the immature alevins that emerged with visible yolk would have difficulty swimming and avoiding predators.

We do not know the effect of long-term exposures to low concentration of NH_3 on time of voluntary emergence, but these tests do produce emergent fry with decreased weight and length. Exposed emergent fry have increased metabolic rate and, therefore, increased demand on yolk reserves and less yolk reserve available for incorporation into developing tissues. Adult trout exposed to NH_3 have increased metabolic rates, and NH_3 probably has the same toxic action in fishes that it has in mammals—impairment of cerebral energy metabolism (Smart 1978).

The lowest concentration of NH_3 that caused fry to be significantly smaller in length and weight at emergence was 2.4 ppb (61- and 40-d exposures) (Figure 2). This concentration is about one-twentieth of the concentration (50 ppb) that caused retardation of growth in rainbow trout fry that had been exposed continuously for about 67 d from the beginning of the egg stage (Burkhalter and Kaya 1977). Pink salmon alevins exposed to NH_3 for 61 d at 4° C in our study were more sensitive (as judged by effects on size) than the faster developing rainbow trout eggs and alevins exposed for about 67 d at 12° C. In the trout study (Burkhalter and Kaya 1977), 25 d of the 67-d exposure were during the egg stage, a stage that is relatively tolerant compared with the alevin stage.

The highest concentrations of NH_3 in the discharge water of a hatchery incubator with an abnormally high density of pink salmon alevins (Bailey et al. 1980) was 0.14 ppb, and the highest concentration from intragravel water of a stream

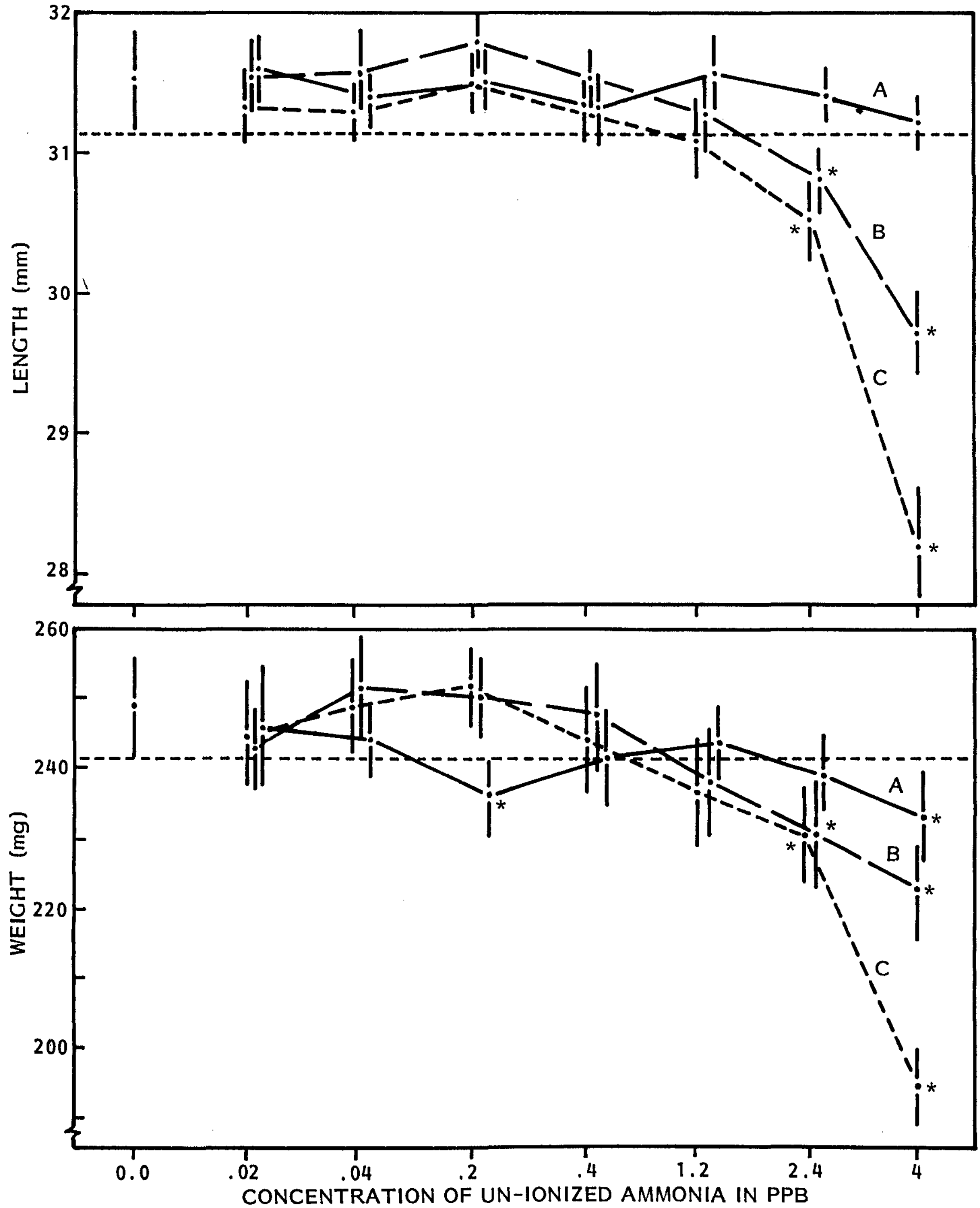

FIGURE 2.—Length (upper) and weight (lower) of migrant pink salmon fry at emergence resulting from groups of alevins exposed to various concentrations of un-ionized ammonia (NH_3) for three lengths of long-term exposure. For each mean, n = 50 fry, bars indicate 95% confidence limits. Group A—exposed for 21 d just prior to migration; group B—exposed for 40 d, ending 21 d prior to migration; group C—exposed for 61 d continuously until migration. Asterisks indicate significant differences in length or weight (P<0.05) between those fry that had been exposed and control fry.

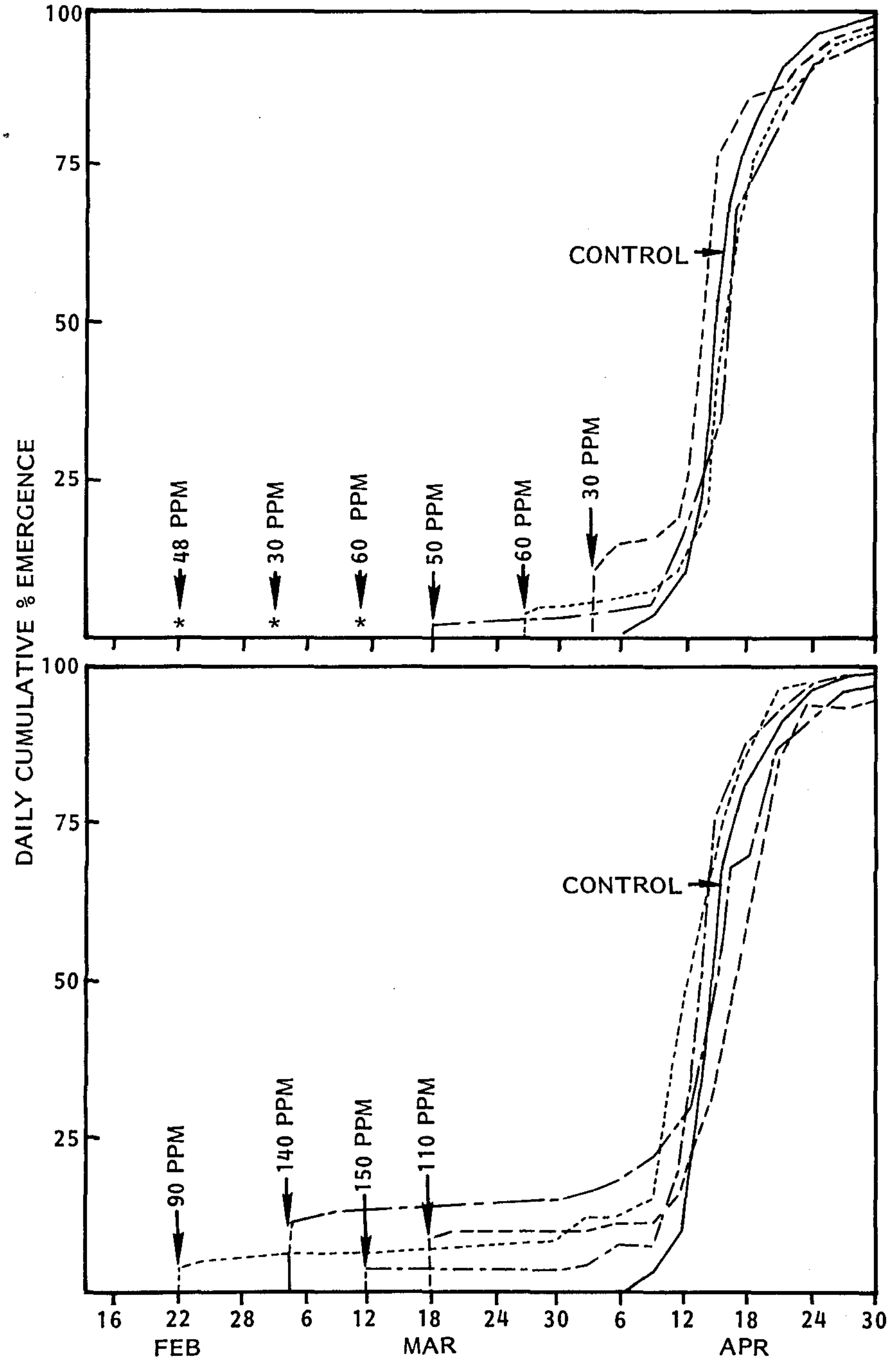

FIGURE 3.—Daily cumulative percent of emergence of pink salmon fry that did not receive exposure to ammonia (controls) or received a single 24-h exposure to ammonia sometime prior to normal emergence. The dates when the 24-h exposures began are indicated by arrows and exposure concentrations. Three ranges of un-ionized ammonia concentrations were used: 1) <25 ppb (not shown) which resulted in <1% immediate emergence. The date of 50% emergence was within ±3 d of 50% control emergence, 2) 30-60 ppb of un-ionized ammonia (upper), 3) 90-150 ppb of un-ionized ammonia (lower). Asterisk indicates the lack of initial emergence (<1%) in response to ammonia exposures (same pattern as controls).

with known densities of pink salmon alevins (Rice and Bailey 1980) was 0.096 ppb. Both of these "real life" extremes of NH_3 were much less than the concentrations we found to cause early emergence of immature fry and acute toxicity. The concentrations that caused small size after lengthy exposure in this study were about 10 times greater than the maximum concentrations found in hatchery incubator effluents and intragravel water from salmon redds (Bailey et al. 1980; Rice and Bailey 1980). Thus, exposure to naturally occurring ammonia is not a likely problem for salmon eggs and alevins in Alaska under natural or hatchery conditions where temperatures are low and waters are acidic—conditions that cause the percentage of NH_3 to be very low (<0.1%).

If pink salmon are reared at higher temperatures and alkalinities, the potential for adverse effects from NH_3 is increased because of the shift in the equilibrium toward NH_3. At 5° C and pH of 7.5, 0.394% of the total ammonia is NH_3; in contrast, at 5° C and pH of 6.5, 0.0395% is NH_3 (Emerson et al. 1975). Therefore, if the pH of Auke Creek and Sashin Creek were 7.5 rather than 6.5, approximately 10 times more NH_3 might have been observed. The level of total ammonia would have been the same, but the percentage of NH_3 would have been much greater. Assuming no losses from aeration or other factors, the percentage of NH_3 would be about 100 times greater at a pH of 8.5 than at a pH of 6.5. It is possible that high densities of alevins in hatcheries or stream gravels could produce unhealthy concentrations of NH_3 if the pH is alkaline. From our exeriments, we conclude that concentrations of NH_3 >0.50 ppb should be avoided. Because our results were generated at relatively low temperature and pH, extrapolation of our data to extreme situations of temperature >10° C or pH >7.8 is inappropriate.

ACKNOWLEDGMENTS

Sidney G. Taylor aided in the 61-d tests and the length-weight analysis. Charlotte Misch conducted the tests on early emergence.

LITERATURE CITED

BAILEY, J. E., S. D. RICE, J. J. PELLA, AND S. G. TAYLOR.
1980. Effects of seeding density of pink salmon, *Oncorhynchus gorbuscha*, eggs on water chemistry, fry characteristics, and fry survival in gravel incubators. Fish. Bull., U.S. 78:649-658.

BAMS, R. A.
1967. Differences in performance of naturally and artificially propagated sockeye salmon migrant fry, as measured with swimming and predation tests. J. Fish. Res. Board Can. 24:1117-1153.
1970. Evaluation of a revised hatchery method tested on pink and chum salmon fry. J. Fish. Res. Board Can. 27:1429-1452.

BURKHALTER, D. E., AND C. M. KAYA.
1977. Effects of prolonged exposure to ammonia on fertilized eggs and sac fry of rainbow trout (*Salmo gairdneri*). Trans. Am. Fish. Soc. 106:470-475.

DOUDOROFF, P., B. G. ANDERSON, G. E. BURDICK, P. S. GALTSOFF, W. B. HART, R. PATRICK, E. R. STRONG, E. W. SURBER, AND W. M. VAN HORN.
1951. Bio-assay methods for the evaluation of acute toxicity of industrial wastes to fish. Sewage Ind. Wastes 23:1380-1397.

EMERSON, K., R. C. RUSSO, R. E. LUND, AND R. V. THURSTON.
1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. J. Fish. Res. Board Can. 32:2379-2383.

EUROPEAN INLAND FISHERIES ADVISORY COMMISSION.
1970. Water quality criteria for European freshwater fish. Report on ammonia and inland fisheries. FAO, EIFAC (Eur. Inland Fish. Advis. Comm.) Tech. Pap. 11, 12 p.

FINNEY, D. J.
1971. Probit analysis, 3d ed. Camb. Univ. Press, Lond., 333 p.

GARRISON, R. L.
1968. The toxicity of pro-noxfish to salmonid eggs and fry. Prog. Fish-Cult. 30:35-38.

PARKER, R. R.
1971. Size selective predation among juvenile salmonid fishes in a British Columbia inlet. J. Fish. Res. Board Can. 28:1503-1510.

PENAZ, M.
1965. Influence of ammonia on eggs and spawns of stream trout *Salmo trutta* M. Fario. Zool. Listy, Folia Zool. 14:47-53. [Translated by and available from Foreign Fisheries (Translations), U.S. Dep. Commer., Wash., D.C.]

RICE, S. D., AND J. E. BAILEY.
1980. Ammonia concentrations in pink salmon, *Oncorhynchus gorbuscha*, redds of Sashin Creek, southeastern Alaska. Fish. Bull., U.S. 78:809-811.

RICE, S. D., D. A. MOLES, AND J. W. SHORT.
1975. The effect of Prudhoe Bay crude oil on survival and growth of eggs, alevins, and fry of pink salmon, *Oncorhynchus gorbuscha*. *In* Proceedings of 1975 Conference on Prevention and Control of Oil Pollution, p. 503-507. Am. Pet. Inst., Environ. Prot. Agency, and U.S. Coast Guard, Wash., D.C.

RICE, S. D., AND R. M. STOKES.
1975. Acute toxicity of ammonia to several developmental stages of rainbow trout, *Salmo gairdneri*. Fish. Bull., U.S. 73:207-211.

SMART, G. R.
1978. Investigations of the toxic mechanisms of ammonia to fish—gas exchange in rainbow trout (*Salmo gairdneri*) exposed to acutely lethal concentrations. J. Fish. Biol. 12:93-104.

U. S. ENVIRONMENTAL PROTECTION AGENCY.
1974. Methods for chemical analysis of water and wastes. U.S. Environ. Prot. Agency, EPA-625/6-74-003, 298 p.

WURTZ-ARLET, J.
1959. Toxicité des detergents anioniques vis-a-vis des alevins de Truite commune [The toxicity of anionic detergents towards the alevins of common trout]. [In. Fr.] Bull. Fr. Piscic. 32:41-45.