EFFECT OF SUSPENDED IRON HYDROXIDE ON THE HATCHABILITY AND EMBRYONIC DEVELOPMENT OF THE COHO SALMON[1]

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Abstract. Coho salmon (*Oncorhynchus kisutch*) eggs were fertilized and fry were incubated at 10°C in the presence and absence of suspended iron. Suspended ferric hydroxide at a concentration of 3 ppm had no apparent effect on the hatchability, embryonic development, or survival and maturation of newly hatched coho salmon still attached to the yolk mass (alevins).

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The deposition of suspended solids on aquatic substrates may result in reduced hatched alevins from spawning areas (Campbell 1954, Cooper 1956). A major source of these suspended materials is the discharge of ferric hydroxide ($Fe(OH)_3$) into streams which has become a major source of pollution throughout the northeastern United States. Ferric hydroxide may be derived from wells and natural springs where iron salts occur in rock strata or from acid mine drainage as a result of the oxidation of iron pyrite (FeS_2). As pH increases, dissolved iron salts will hydrolyze to ferric hydroxide and precipitate as a yellow-brown slime on the bottom of streams. This precipitate has been shown to cause a variety of physiological changes (Brenner *et al* 1977), including acute respiratory distress in fish (Larson and Olsen 1950), or affect the development, survival, growth, and reproduction of invertebrates and fish on a long-term basis (Sykora 1970, Sykora *et al* 1972a, Sykora *et al* 1972b, Sykora *et al* 1975, Smith *et al* 1973, Smith and Sykora 1976). The effect of iron hydroxide on the embryonic development of the coho salmon (*Oncorhynchus kisutch*) was investigated using facilities at the Pennsylvania Fish Commission's fish culture station in Tionesta, PA.

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METHODS AND MATERIALS

Coho salmon eggs were received from the Pennsylvania Fish Commission's Walnut Creek station, Erie, PA, by the Tionesta station on 8 and 11 November 1973. Eggs were collected and fertilized at the Walnut Creek Station and transported by the Pennsylvania Fish Commission to the Tionesta station, and the time interval between fertilization and beginning of incubation at 10°C was less than 24 hrs in all cases.

The different development stages (as defined by Zeitoun and Tack, 1974 and Brenner *et al*, 1976) were identified by removing 10 eggs from the hatching jars at 5-day intervals until the eye-up stage, with 5 being placed in Kahl's fixative and 5 frozen at −20°C. Frozen eggs were analyzed for total iron by atomic absorption spectrometry. Fixed specimens were dehydrated in alcohol and xylol and prepared as whole mounts on transverse sections. Toluene was used as a clearing agent before infiltration of the embryo (Guyer 1936). Embryos were embedded in Tissuemat (Fisher) at 52.5±0.5, sectioned at 8 μ, stained with hematoxylin counter stained with eosin and mounted in Canada balsam. Whole mount embryos, after removal of the told sac, were stained with alum cochineal solution.

The hatchability of eggs was determined by the removal and counting of dead eggs daily during incubation. Dead alevins were also removed from the rearing tanks and recorded daily until 90 days of age. After 90 days, alevins from both groups were mixed in rearing ponds and further observations on survival were not continued.

Only eggs received from Walnut Creek Station were used in the embryonic development study to assure that all test specimens were obtained from a common source. Ova from Michigan (Dept. of Environmental Resources) were observed only for hatchability and alevins mortality. Upon arrival at Tionesta Station,

TABLE 1
Mean and 95% Confidence Intervals of Five Water Chemistry Parameters from Filtered and Unfiltered Samples.

Parameters	Filtered (Control)			Unfiltered (Experimental)		
	No. Detn.	Mean	95% Conf. Interval	No. Detn.	Mean	95% Conf. Interval
pH	5	7.8	0.11	5	7.5	0.12
Hardness*	5	70.0	3.20	5	75.0	3.40
Dissolved Oxygen	50	10.4	0.98	50	10.2	0.88
(% Saturation)		(91.6)			(91.6)	
Temp. °C.	50	10.1	1.00	50	10.0	1.00
Iron**	5	0.1	0.02	5	3.3	0.41

*Hardness as EDTA mg $CaCo_3^{-1}$.
**Iron as mg Ferric Hydroxide/ℓ^{-1}.

the eggs were divided into 2 groups: one group received water (10°C) directly from a well which contained 3 ppm iron hydroxide and the second group received water which was passed through a sand filter which effectively removed the suspended iron hydroxide. Eggs were incubated in 3.8 liter non-toxic polystyrene hatching containers with at least 3 replicates of 10,000 eggs per container. Water flow was maintained at 2.3 liters per minute per container and the mean dissolved oxygen content was 10 ppm (91.6% saturation). Water from both the filtered and unfiltered sources was analyzed periodically for hardness (EDTA $CaCO_3^{-1}$) and pH by standard methods (Amer. Pub. Health Asso. 1965). Temperatures were recorded daily and the dissolved oxygen concentrations as determined by the modified Winkler method (Lee and Stumm 1960). The total ferric concentration was determined by a modification of Shapiro's (1965) procedure for atomic absorption spectrometry. Chemical analysis, according to the procedures outlined in the EPA Methods of Water and Wastes (1974), indicated that except for the iron concentration, the water received by both groups of eggs was similar (table 1).

RESULTS

The sequence of development was the same in embryos incubated in the presence or absence of 3 ppm suspended iron (fig. 1). Suspended iron also did not cause any discernible effect on the appearance of the major systems, as indi-

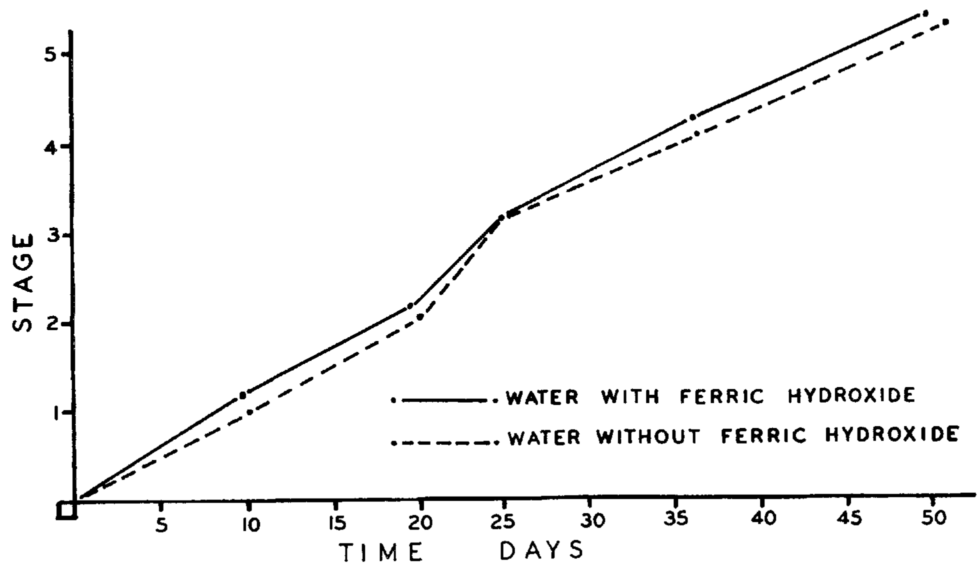

FIGURE 1. Rate of development of coho salmon (*Oncorhynchus kisutch*) at 10°C. The stages of development correspond to those described by Zeitoun and Tack (1974) and Brenner *et al* (1976).

TABLE 2

Length of developmental period, % hatch and number of thermal units required for development of coho salmon (Oncorhynchus kisutch) *incubated at 10°C in the presence and absence of 3 ppm ferric hydroxide.*

	Ferric Hydroxide	Ferric Hydroxide Absent	
Location of Eggs	Walnut Creek	Walnut Creek	Michigan
Number Eggs/Sample	30,000	30,000	600,000
Average Number Eggs/Jar	10,000	10,000	10,000
Water Temperature °C	10°	10°	10°
Days until Eye-Up	26	24	24
% Eye-Up	72	68.5	67.5
Thermal Units at Eye-Up °C	218	201	201
Days required for Hatching	51	53	53
% Hatch of Eye-Up Eggs	96	99	99
% Hatch of Total Eggs	66	67	67
Thermal Units at Hatching °C	428	445	445

cated by histological examination. Likewise, iron was not detected in either group of eggs when analyzed by atomic absorption spectrometry. The eye-up stage is used by hatchery personnel to determine the number of viable eggs present in the incubator at the approximate halfway point in development. Eye-up occurred in 26 days with 3 ppm iron and 24 days for eggs incubated at 10°C without suspended iron. The expected number of eye-up eggs based on previous experience with coho salmon at the Tionesta station was between 60–70%. The eye-up percentage did not vary significantly from the expected on the basis of a Chi square analyses among the various groups of coho eggs, regardless of the environmental condition ($P<.50$). Similarly, the number of thermal units (0.56°C (1 df) <0°C (32 df)) required for eye-up also did not vary significantly among the different groups of eggs (table 2). Moreover, there was no significant difference in hatchability between the different groups of eggs, nor did the presence of ferric hydroxide affect the survival of viable eggs. The percent of alevins hatching from eye-up eggs was essentially the same for both groups of eggs incubated at 10°C.

The developmental period after hatching was divided into 3 stages: sac alevins, swim-up alevins and feeding alevins. Feeding occurred at 22 days and 21 days for coho salmon fry incubated in the presence and absence of suspended iron, respectively. Mortality of fry over a 3 month period did not vary significantly between the 2 experimental treatments (table 3).

DISCUSSION

Previous studies have indicated that a similar pattern of development occurs in the Salmonidae although the length of the developmental periods and temperature preference vary among the species (Battle 1944, Riddle 1917, Mahon and Hoar 1956, Zeitoun and Tack 1974, Knight 1963, Ballard 1973a, b, c). In this regard, Embody (1934) concluded that development occurs linearly over the normal temperature range for a particular species, with departures from linearity at both the upper and lower extremes. Our results indicate that a concentration of 3 ppm of ferric hydroxide had no apparent effect on the rate of development or mortality of embryonic coho salmon. Smith and Sykora (1976) reported a mean hatch of 69% and 53% for coho salmon incubated in 3 ppm suspended iron compared to a 67% hatch observed in our study. Furthermore, these authors reported a 58% survival of 90-day old alevins in their control and a 58% and 66% survival of those incubated in 3 ppm suspended iron. These results are considerably less than

TABLE 3

Mortality and development of coho salmon (Oncorhynchus kisutch) *alevins which were incubated at 10°C in the presence and absence of 3 ppm ferric hydroxide.*

	Ferric Hydroxide	Ferric Hydroxide Absent	
Location of Eggs	Walnut Creek	Walnut Creek	Michigan
Number of Sac-Fry	19,800	20,100	402,000
% Mortality	0.1	1.0	1.0
Water Temperature °C	10	10	10
Age at Feeding (Days)	22	21	21
Number of Swim-Up Alevins	19,780	19,899	379,980
% Mortality	0.0	0.0	0.0
Water Temperature °C	10	10	10
Number Feeding Alevins	19,780	19,899	397,980
% Mortality	6.0	3.0	3.0
Water Temperature °C	11	9	9
Total % Mortality	6.1	4.0	4.0
Number Alevins Surviving 90 Days	18,593	19,183	386,041

the 93.9% and 96.0% survival of our control alevins. Sykora *et al* (1972a, b) demonstrated that suspended iron concentrations in excess of 6 mg Fe/liter impaired the growth of brook trout (*Salvelinus fontinalis*) and they assumed that reduced visibility, due to high turbidity, prevented the fish from feeding and resulted in a reduced growth rate. Smith *et al* (1973) reported that an iron hydroxide concentration of 1.5 mg Fe/liter reduced the hatchability and growth of fathead minnows (*Pimephales promelas*) whereas the hatchability of brook trout eggs was unaffected in iron hydroxide concentrations from 0.75 to 12 mg Fe/ℓ^{-1}. Similarly, a concentration of 3 ppm ferric hydroxide had no apparent effect on the hatchability, embryonic development or survival and maturation of coho salmon alevins. It is doubtful that coho salmon would spawn in a stream containing iron hydroxide since Updegraff and Sykora (1976) reported that the species will avoid the pollutant regardless of whether they were previously exposed to iron hydroxide for several months prior to exposure. Therefore, iron hydroxide, in concentrations exceeding 3 ppm, may adversely affect coho salmon even through a concentration of 3 ppm did not appear to reduce hatchability or increase embryonic mortality.

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