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EXPLORATORY STUDIES OF THE INTERACTIONS
OF GAS SUPERSATURATION AND TEMPERATURE
ON MORTALITY OF JUVENILE SALMONIDS¹

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9-28-70

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28 September 1970

For review by Technical Advisory Committee and participants in
the Columbia River Thermal Effects Study.

¹Research conducted under U. S. Atomic Energy Commission Contract AT(45-1)-1830.
This study was coordinated with the interagency Columbia River Thermal Effects
Study.

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INTRODUCTION

This report constitutes a preliminary summary of several experiments. It is intended to provide information to the participants and Technical Advisory Committee of the Columbia River Thermal Effects Study without constituting a manuscript intended as suitable for publication in a technical journal. Conclusions presented are tentative and subject to revision at such time as the experiments can be adequately repeated.

Recent studies by the U. S. Bureau of Commercial Fisheries, Seattle Biological Laboratory, (BCF) showed a seasonal cycle of supersaturation of dissolved nitrogen in the Columbia River between Grand Coulee Dam and the estuary (W. J. Ebel, 1969). The dissolved nitrogen increased from fall and winter levels near 100% air saturation to supersaturation levels (often above 135%) in spring and summer. The dissolved nitrogen is apparently correlated with flow of water over spillways of dams where (as shown by Harvey and Cooper (1962) on Corbald Creek, B. C.) nitrogen supersaturation apparently results from water cascading into plunge basins.

Adverse effects on fish of high gas saturation values have been known for some time Coutant and Genoway (1968). The "gas bubble" disease has generally been considered to be mostly a physical phenomenon due to differences between the sum of the individual tensions of the gasses dissolved in water and the hydrostatic pressure (Doudoroff 1957). A drop in hydrostatic pressure was shown to induce bubble formation (embolism) in the fish tissues. It has been recognized more recently that chronic high gas saturations such as exist seasonally in the Columbia River also create gas embolisms and inflict heavy mortalities on anadromous fishes.

Symptoms of this disease have been recognized both in adult and young fish. They include a general darkening of coloration and visible bubbles forming in fins, on the head, around the eyes, and in membranes of the mouth, gills and opercula. Affected fish become restless and easily disturbed. Aimless swimming punctuated by rapid bursts is followed by a period of quiet before death. Very small fish become buoyed to the surface by large internal bubbles. Secondary infections of damaged tissues by bacteria and fungi may contribute to death. Transfer of affected fish to water at air equilibrium generally provides recovery if primary gas bubble symptoms are not too advanced.

The influence of water temperature rise on the formation of supersaturated gas conditions was recognized by Engelhorn (1943) and was used by him to create test situations. In these tests, fish exhibited gas bubble disease symptoms similar to those that developed from artificial pressure changes. Rucker and Hodgeboom (1953) report that warming of hatchery supply water originally at sublethal saturation values of nitrogen resulted in higher saturation levels and gas bubble disease. Westgard (1964) noted temperature-induced gas pressure changes in water supplies to a fish spawning channel, but attributed most of the observed supersaturation of nitrogen to cascading inflow water into a narrow pit spillway.

Recently, concern has been expressed over the potential for induction of "gas-bubble" disease in anadromous fish which must pass through varying temperature during migration (Ebel, 1969 and personal communication). Abrupt differences between external and internal gas tensions may be created as fish change temperature while they enter tributary streams, change depths at fish passage facilities at dams or pass through mixing zones below thermal discharges such as at Hanford. In addition, river temperature levels considered safely below the upper limits of thermal tolerance for the fish species may induce mortalities through interaction with elevated or changing gas saturations.

The principal objective of these exploratory studies was to determine, in a preliminary manner, the levels of gas supersaturation and temperature change required to produce identifiable synergistic effects in mortality of juvenile salmonids. The studies were directed towards specific questions arising from the passage of outmigrants through Hanford thermal plumes. Maximum temperature increases within the largest of the plumes are illustrated in Figure 1. Fish pass through this fluctuating thermal regime at approximately 1 meter per second. The two questions examined were 1)

whether resistance (survival) times of juvenile salmonids to acute thermal shock are shortened by simultaneous high levels of gas supersaturation, and 2) what duration of brief exposure to simultaneous temperature rise and elevated gas supersaturation is required to induce immediate or delayed mortality of juvenile salmonids acclimated to conditions extant during spring out-migration? None of the studies reported here are definitive; they are presented as guides to the participants in the Columbia River Thermal Effects Study to further research and to preliminary assessments of environmental hazards of thermal additions.

ACUTE THERMAL SHOCK OF JUVENILE CHINOOK SALMON

Methods and Materials

The objective of this series of experiments was to determine if resistance times of juvenile Chinook salmon to acute thermal shock would be shorter with simultaneous high gas saturation than with thermal stress alone. The saturation levels used were those which resulted from heating air-equilibrated river water to the desired test temperature.

The environmental situation which was roughly simulated in these experiments was a thermal mixing zone ("plume") in which juvenile salmonids migrating downstream at essentially the rate of current flow can be drawn involuntarily into zones of elevated temperature. In such a plume, fish experience a sharp rise in temperature, followed by decreasing temperatures until the mixed river temperature is reached. Gas supersaturation likely follows the same pattern since time is not available for gas equilibration with the atmosphere. Jaske, Templeton and Coutant (in press) have demonstrated the utility of thermal resistance data at constant temperatures for predicting thermal death in this fluctuating thermal regime. Gas supersaturation may be suspected of providing a double stress during exposure of fish to lethal temperatures, even when the fish are not suffering from chronic supersaturation effects upstream of effluents.

Juvenile Chinook salmon were raised in air-equilibrated water at the aquatic ecology facilities of the Pacific Northwest Laboratory from eggs obtained at Priest Rapids Dam, courtesy of the Washington State Department of Fisheries.

At time of testing in early July 1969, fish lengths and weights averaged 74.70 MM (S.D.=7.68) and 6.69 g (S.D.=1.78), respectively.

Geometric mean times to loss of equilibrium (complete body inversion) and death (cessation of opercular movement) were determined for groups of 10 to 20 fish at selected lethal temperatures, 26, 27, 28, 29 and 30C. The

experimental method was similar to that of Brett (1952) except that running water (about 0.3 l/sec.) at the desired temperature was supplied to the test chamber (0.18 m³ of water) which was open to the atmosphere. Shock temperatures were maintained within 0.05C by a stream heat exchanger and temperature controller. Supersaturated gasses were removed from inflowing water in low gas tests with a compressed air gas scrubber (Coutant and Genoway, 1968). When high gas saturations were desired, no compressed air was introduced. A test was conducted at 30C at an intermediate gas concentration. Determinations of dissolved nitrogen content of test water (table 1) were made on a Loenco Model 15B gas chromatograph, with air calibration. Percentage of air saturation was calculated from gas concentration, temperature and barometric pressure. Accuracy was estimated to be ± 5 percent.

Results

Geometric mean resistance times for equilibrium loss and death (with 95% confidence limits) are shown in figures 2 and 3, respectively. At nearly each temperature, the confidence limits of the geometric means overlap. The exception was shown by the death times at 30C, especially when resistance at the highest gas saturation is considered. There was, therefore, no consistent, statistically significant decrease in thermal tolerance when elevated gas saturation is present as a potential second stress. Regression analyses of equilibrium loss and death times reveal closely similar lines, which cross near 26.5C. Fish tested above that temperature exhibited slightly increased tolerance to thermal stress in the presence of high gas supersaturation. Below 26.5, tolerance was decreased. Neither changes were statistically significant, however, in the range of temperatures examined. This was true whether physiological death or equilibrium loss was considered as the end point.

Discussion

The physiological mechanism of thermal death of fishes is incompletely understood, but one hypothesis suggests that oxygen delivery to nervous tissue lags behind metabolic usage at the lethal temperatures. Oxygen supersaturation in the medium would theoretically provide additional oxygen supplies and allow longer survival. The results of the present resistance experiments would lend further credence to this hypothesis, although additional study is needed.

It is important that the fish tested were not already affected by chronic supersaturation effects, but were healthy specimens derived from stock tanks maintained near air equilibration. This simulates passage of downstream migrants in the river before active spilling of runoff waters at upstream reservoirs and development of consequent chronic high gas saturation. Weakened fish with high gas levels in their tissues may react differently than the fish in this experiment.

DOSE EXPERIMENTS

Methods and Materials

In these experiments, young rainbow trout were first acclimated to conditions of temperature (10C) and gas saturation (115% N_2) typical of the central Columbia River during the principal period of seaward emigration of juvenile salmonids (Ebel, 1969). These conditions were not lethal or obviously debilitating during the three-week acclimation period, but they were intended to yield fish already stressed by supersaturated gasses.

Following acclimation, fish were given timed exposures to elevated temperatures and gas saturations expected at those temperatures (from gas-solubility/temperature relationships) and were returned to the conditions of acclimation. Mortalities during the exposures were recorded, as were delayed

mortalities occurring for several days thereafter (over 30 days in some instances). Temperatures and durations of exposure were selected that were reasonable for the temperature-flow regimes of the Hanford K-plume (Figure 1). The conditions were held constant, however, rather than simulating the exponential decreases in temperature (and, presumably, N_2 saturation) in the plume. These experiments were, therefore, more stressing than the conditions existing in the field.

Fish were acclimated in hatchery troughs 152 cm long, 30 cm wide, with a water depth of 16 cm. A continuous flow of about 0.3 liter per second was maintained through the trough. Following exposures, controls and shocked fish were held either in the same troughs, now subdivided, or in 20 cm diameter liveboxes placed in an 18-meter diameter fiberglass tank holding circulating water about 50 cm deep. Temperature and gas supersaturation were maintained by mixing heated and unheated river water at the trough or tank inlets.

Test exposures were made in a plexiglass trough 70 cm long, 25 cm wide containing 15 cm of water. A plastic basket held the fish within the trough, and facilitated rapid transfer of fish. A continuous water flow of about 0.3 liters per second was maintained through the trough. Temperature was controlled by a mixing valve blending heated and unheated river water. Supersaturation of nitrogen gas was created in the heating process, but could be controlled with an air scrubber. Determinations of dissolved nitrogen content of the water, and percentage of atmospheric saturation, were made as described in the preceeding experiment.

Results

The initial experiment consisted of a 5 deg.C rise in temperature to 15C and a consequent rise in gas saturation from about 115% to about 128% (due to temperature-gas solubility relationships). Gas saturation during the

exposures varied considerably as shown by results of analyses made after the test exposures had been completed. Sixteen samples ranged from 111% to 142% (mean=124%, s.d.=10.3). The time lag between obtaining a water sample and receipt of the results precluded more careful adjustment of exposure conditions.

Cumulative mortalities during about one month holding after return to acclimation conditions are shown in Figure 4. Noticeable deviation of mortalities from the control occurred from exposures of 8 min. and greater, although 50% mortality did not result from any of the exposures administered. A gas saturation value of 142% was obtained during the 8, 16 and 32 min. exposures, suggesting more stressing conditions than were intended.

A second experiment involved a 10 deg.C temperature rise (to 20C) and a consequent rise in gas saturation to about 140%. Again, there was variation in the gas saturation conditions among 13 samples, with a range of 128% to 149% (mean 138%, s.d. 7.4). The results (Figure 5) were somewhat erratic, but indicate significant mortalities after exposures of 360 min. The 50 percent mortality level was not reached, however, after any exposure. Complete resistance time data were obtained for one group of 25 fish held in the test conditions until mortality. The median death time was 8 hr. although 100 percent mortality did not occur until 28 hr.

Discussion

Despite the considerable time required to conduct these tests, and to maintain the fish following test exposures, the results are less than satisfactory for defining the dosages of elevated temperature (and consequent elevations in gas saturation) required to inflict significant mortality upon populations of young trout. It would appear that less time

was required for mortality under the less severe conditions of 15C and about 120% saturation than was required under the more severe conditions. This is at variance with theoretical considerations.

While the response pattern remains unclear, the tests did contribute to an understanding of processes occurring in the Hanford thermal plumes. At a rate of passage through a plume (Figure 1) approximately 0.5 to 1 m/sec, juvenile migrants would be exposed to elevated temperatures greater than 3 deg.C, (and also elevated gas saturations at those points) for only a few minutes. The resistance pattern obtained at 20C and 140% suggests that direct mortality requires a matter of hours of exposure, rather than minutes. Significant latent mortality occurred only after 360 minutes (6 hr).

The first experiment yielded less reassuring results. Eight, sixteen and thirty-two minute exposures appeared to induce mortality. These three exposures were suspect, however, since the fish received gas saturations of 142% during part of the test duration. A duration of 447 min., however, did not induce significant mortality and 1889 min. (31 hr., 29 min.) induced only 24% mortality as recorded for a month following the exposure. These are considerably longer than travel time through a plume.

Thus, it seems reasonable to conclude that fish would not experience stresses from temperature-induced gas supersaturation in the Hanford plumes that would cause significant mortalities, either immediate or at a 30-day period after the experience.

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FIGURES

Figure 1: Columbia River surface temperature patterns in the mixing plume of the KE and KW reactor discharges at low-flow conditions (41,000 cfs).

Figure 2: Geometric mean times (and 95% confidence limits) to equilibrium loss of juvenile chinook salmon tested in water with high and low gas supersaturation.

Figure 3: Geometric mean times (and 95% confidence limits) to death of juvenile chinook salmon tested in water with high and low gas supersaturation.

Figure 4: Cumulative mortalities of juvenile rainbow trout during holding after timed exposures to 15C and about 120% gas saturation.

Figure 5: Cumulative mortalities of juvenile rainbow trout during holding after timed exposures to 20C and about 140% gas saturation.

Table 1. Gas saturation values during thermal resistance experiments.

<u>Temp.</u>	<u>High Gas</u>		<u>Low Gas</u>
26	135		106
27	142		119
28	146		116
29	120		113
30	147	*135	106

* intermediate gas saturation

FIGURE 1. Columbia River surface temperature patterns in the mixing plume of the KE and KW reactor discharges at low-flow conditions (41,000 cfs).

COLUMBIA RIVER SURFACE TEMPERATURE PATTERNS

41,000 cfs Flow

*Detection Limit of Aerial Infrared Imaging System $\sim 0.5^{\circ}\text{C}$

Figure 2. Geometric mean times (and 95% confidence limits) to equilibrium loss of juvenile chinook salmon tested in water with high and low gas supersaturation.

EQUILIBRIUM LOSS TIMES OF JUVENILE CHINOOK SALMON IN HIGH AND LOW N₂ SUPERSATURATION - CCC-2/70

PNC 10

FIGURE 3: Geometric mean times (and 95% confidence limits) to death of juvenile chinook salmon tested in water with high and low gas supersaturation.

DEATH TIMES OF JUVENILE CHINOOK SALMON IN HIGH AND LOW N₂ SUPERSATURATION - CCC 2/70

FIGURE 4: Cumulative mortalities of juvenile rainbow trout during holding after timed exposures to 15C and about 120% gas saturation.

Figure 4

0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36

DAYS FOLLOWING TEST EXPOSURE (EXPOSURE ON DAY 0)

CUMULATIVE MORTALITY (%)

FIGURE 5. Cumulative mortalities of juvenile rainbow trout during holding after timed exposures to 20C and about 140% gas saturation.

1.1

JUVENILE DAINBOY (BOAT)

EXPOSURE TIME (MIN)

UNTIL 12.4

0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88 92 96 100

360

30

33

EXPOSED TO 20°C AND ABOUT 10% SAT. N₂

ACCLIMATED AND HELD AT ABOUT 10°C AND 11.5% SAT. N₂

SURVIVAL AT TERMINATION (%)

0

80

96

96

17

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$$(a_7 a_6 \dots a_0)_{10}$$

76

78

02

28

2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
DAYS FOLLOWING EXPOSURE (EXPOSED ON DAY 0)																	