THERMAL TOLERANCE OF JUVENILE PACIFIC SALMON AND STEELHEAD TROUT IN RELATION TO SUPERSATURATION OF NITROGEN GAS

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ABSTRACT

Thermal tolerance of juvenile chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*), and steelhead trout (*Salmo gairdneri*) that had been held at various acclimation temperatures was lowered when test water was supersaturated (125-130% of saturation) with nitrogen gas. Increasing the depth of the test tank allowed the fish to compensate somewhat for the supersaturation by sounding, but substantial mortalities still occurred. A comparison of tolerance among the species tested revealed that coho salmon were the most tolerant, chinook salmon next, and steelhead trout the least tolerant to temperature increases in the presence of supersaturation of nitrogen.

During the past several decades, a number of investigators have examined temperature as a lethal factor by use of the classic pharmacological assay method. Fry, Hart, and Walker (1946) recognized the importance of acclimation temperature in determining the tolerance of a fish to high and low temperatures and established upper and lower levels of tolerance at various acclimation temperatures. Brett (1952) listed maximum temperatures for survival of young Pacific salmon (*Oncorhynchus* spp.) between 23.8 and 25.1° C. In later work (Brett, 1958), he emphasized temperatures below those at which a fish dies and constructed hypothetical temperature polygons which described lower levels of temperature tolerance where activity, growth, and spawning would be affected.

More recently, investigators have emphasized the response of fish to temperature changes under multivariate conditions. Many factors such as dissolved oxygen deficits, carbon dioxide increases, and increases in toxic substances all affect an aquatic organism's tolerance to temperature increases. Mihursky and Kennedy (1967) stressed the importance of multivariate experiments for establishing more realistic standards for temperature regulation.

Several nuclear power plants have been proposed for the Columbia River. The National Marine Fisheries Service (NMFS) is particularly concerned about the effect that heated effluents from these plants might have on juvenile Pacific salmon and steelhead trout (*Salmo gairdneri*) migrating downstream, particularly while they are stressed by supersaturated nitrogen gas. High levels of nitrogen gas (over 125% saturation) occur within large areas of the Columbia from about early May until mid-August (Ebel, 1969). This period coincides with the downstream migration of most juvenile salmon and trout. Although the effect of supersaturation of gas on juvenile salmon and trout has not been examined in great detail, preliminary studies by Ebel (1969) clearly show that Columbia River juvenile salmon have considerably lower tolerance to temperature increases when stressed by supersaturation of nitrogen than the tolerance indicated by Brett (1952).

The Federal Water Quality Administration (FWQA) recognized that supersaturation of dissolved nitrogen could be a significant factor in establishing water quality criteria for the Columbia River. It therefore contracted the Bureau of Commercial Fisheries (BCF; presently designated as NMFS) to determine the changes in tolerance of juvenile salmon and trout to temperature increases at different levels

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of temperature acclimation and supersaturation of nitrogen. In the experiments described in this report, BCF personnel sought to determine the change in tolerance of juvenile salmon and trout to temperature increases when stressed by supersaturation of nitrogen and to determine possible changes in tolerance if they had the option to sound to different depths. Ebel (1969) reported that the depth at which fish migrate influences the effect of supersaturation of nitrogen because the gas remains in solution at a much higher concentration when under pressure; we therefore considered depth as well as temperature increase and supersaturation of nitrogen to be important.

Our first series of experiments describe the effect of supersaturation of nitrogen and temperature increases at surface pressures. Later experiments show how depth changes the above effect.

METHODS

The general approach used to determine the effect of nitrogen supersaturation on the tolerance of juvenile salmon and trout to increased temperature was similar to that used by Brett (1952). Stocks were acclimated to temperatures identical to those used by Brett; test temperatures encompassed the ranges Brett used in his lethal temperature determinations. These test and acclimation temperatures were purposely selected so that changes in tolerance caused by the stress of supersaturated nitrogen could be compared with Brett's well-established levels of temperature tolerance.

Groups of 20 test fish, each acclimated to a given temperature, were placed simultaneously in control and test situations involving identical test temperatures at treatments of high (125-130%) and normal (100%) nitrogen saturation. Two acclimation groups were used in each set of tests—one that had a normal acclimation history and one that had been exposed to supersaturated nitrogen for 720 min. Observations of behavior and mortality were made continuously for the first 6 hr, then every hour for the remainder of an 18-hr period. Events recorded were times to first indication of stress, to loss of equilibrium, and to death. The numbers of live and dead fish with obvious external symptoms of gas bubble disease were recorded at the termination of tests. All tests were then repeated (most tests were duplicated, some were done three times); the data given in this report are derived from the average value of the duplicated tests.

Hatchery and wild stocks of fish were tested. Hatchery fish were from the following stocks: coho salmon (*O. kisutch*) reared at Leavenworth National Fish Hatchery, Leavenworth, Wash.; spring chinook salmon (*O. tshawytscha*) reared at Little White Salmon National Fish Hatchery, White Salmon, Wash.; and steelhead trout reared at the Washington State Fish Hatchery at Green River, Cumberland, Wash. The wild fish (spring chinook salmon) were collected from the turbine intake gatewells at McNary Dam on the Columbia River. Because of time limitations and lack of sufficient populations of fish, only the coho were tested through the entire range of acclimation temperatures (5, 10, 15, and 20° C). Hatchery-reared steelhead trout and hatchery and wild spring chinook were tested only after exposure at selected acclimation temperatures—steelhead at acclimation temperatures of 10° and 15° C and wild chinook at 10° C.

The experimental temperatures (test and acclimation), lengths of time that the various groups of fish were in holding and acclimation tanks, and size of fish at time of testing are summarized in Table 1. Water in test tanks was adjusted to the appropriate test temperatures established for each acclimation temperature. Temperatures were maintained within ±0.2° C in both test and acclimation tanks. When the test series required stressing of the acclimated fish with supersaturated nitrogen, an acclimation tank at the appropriate temperature was saturated at 115 to 120% nitrogen and about 114 to 120% oxygen. The fish were then transferred from the normally saturated tank (100%) to the supersaturated tank and stressed for 720 min. When supersaturated water was needed in the test tanks, the supersaturating equipment was activated, and each tank was adjusted to maintain between 125 and 130% nitrogen. To ensure stability, the temperatures and satura-

TABLE 1.—Holding time and temperature of water before transfer to acclimation tanks, acclimation conditions, and mean size at time of testing of three species of salmon and trout.

Species and origin of fish	Time in holding tank	Water temperature in holding tank	Acclimation conditions		Mean size of fish at time of test	
			Days	Temperature	Length	Weight
	days	*° C*		*° C*	*mm*	*g*
Hatchery coho	28	8.8	35	5.0a	117	17
	28	8.8	17	10.0a	118	18
	28	8.8	23	10.0	134	22
	28	8.8	4	12.5	134	22
	28	8.8	48	15.0a	134	22
	28	8.8	3	11.7	118	18
	28	8.8	4	14.0	118	18
	28	8.8	16	15.0	118	18
	28	8.8	4	17.5	118	18
	28	8.8	3	20.0a	118	18
Wild spring chinook	0	15.0	4	10.0a	129	19
Hatchery spring chinook	90	8.8-13.0	4	15.0a	134	23
	90	8.8-13.0	3	15.0	134	23
	90	8.8-13.0	3	17.0	134	23
	90	8.8-13.0	3	20.0a	134	23
Hatchery steelhead	10	12.0	5	10.0a	179	54
	10	12.0	3	14.0	179	54
	10	12.0	42	15.0a	179	54

a Final acclimation temperature.

tion levels were then measured for 24 hr before introduction of the test fish.

The source of supersaturation of nitrogen gas in the Columbia River was entrained air. We used pumps to entrain air in our test and acclimation tanks. Oxygen, therefore, was also supersaturated; the ratio of oxygen to nitrogen supersaturation was similar to that recorded in the river (Beiningen and Ebel, 1971). Oxygen saturation varied from about 114 to 120%.

Facilities used for tolerance tests in relation to supersaturation of nitrogen at surface pressures were designed to provide a continuous supply of fresh water. Sufficient cooling and heating capacity was available from a water heater and chiller to continuously supply 227 liters/min of either heated or chilled water from 5° to 40° C. Ten cylindrical fiber glass acclimation tanks, about 1 m high × 2½ m in diameter, provided sufficient space to maintain 2000 fish at each of five acclimation temperatures without crowding. The test tanks were rectangular fiber glass tanks of 113.6-liter capacity. A flow of approximately 11.5 to 30.0 liters/min was maintained in acclimation tanks and 3.5 liters/min in test tanks.

Supersaturation of nitrogen and oxygen was achieved in the acclimation tanks by metering air into the intake of two high-pressure recirculating pumps with 42 kg/cm^2 back pressure on the discharge side of the pump. About 0.75 liter/min air in each pump created the desired saturation of nitrogen (115-120%). Supersaturation of nitrogen and oxygen in the test tanks was achieved by metering 0.05 liter/min into another high-pressure recirculating pump with 32 kg/cm^2 back pressure, which recirculated the water in the coldwater supply tank to the test tanks. (Source of water was from Seattle municipal supply; chlorine was eliminated by charcoal filters.) The final saturation value for each tank was then achieved by manipulating the number of equilibrating screens through which the water flowed before entering the tanks.

The test tank (9 m deep) used for our experiments concerning water depth has been described by Pugh, Groves, and Ebel (1969). When tolerance tests were conducted in the deep tank, the saturation levels of nitrogen and oxygen were controlled in the above manner (by injecting air into a recirculating pump). Because of the large volume of water (66,648 liters), a continuous flow of fresh water was not needed and the existing water was recirculated. Time limitations precluded testing more than two populations (hatchery coho and wild spring chinook salmon) at 10° C acclimation temperature.

EFFECT OF SUPERSATURATION OF NITROGEN GAS ON TOLERANCE OF FISH TO TEMPERATURE INCREASES

Supersaturation of nitrogen (125-130%) in the test tanks lowered the tolerance of all fish to temperature increases at all acclimation temperatures when tested below 26° C (Tables 2-10). More than 50% mortality occurred within 18 hr even with no temperature increase when the test tanks were supersaturated at this level. Time to death of fish was accelerated regardless of acclimation temperature.

A comparison of LE_{50} (exposure time when 50% of the population is dead) curves developed under the four treatment levels indicates that a prior stress of 115 to 120% saturation for 12 hr did not greatly affect the fish when they were subjected to temperature increases in water saturated at 100% (Figure 1). When these

TABLE 2.—Mean values[a] of lethal exposure time (in minutes) for coho salmon that had been acclimated to 5° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		5	10	15	20	23	25
One fish	NN	--	--	--	--	125	15.5
	NS	525	412	380	230	85	14
	SN	--	--	--	--	102	8
	SS	250	330	197	112	35	10
Three fish	NN	--	--	--	--	167	20.5
	NS	615	457	405	270	108	16.5
	SN	--	--	--	--	270	12
	SS	483	435	315	128	137	13
Half of sample	NN	--	--	--	--	300	27
	NS	810	605	577	390	266	21.5
	SN	--	--	--	--	--	20
	SS	626	580	435	229	186	20
All of sample	NN	--	--	--	--	--	64
	NS	--	990	--	1,080	420	58
	SN	--	--	--	--	--	60
	SS	--	--	--	840	450	445

a Mean of replicated tests.
b NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

TABLE 3.—Mean values[a] of lethal exposure time (in minutes) for coho salmon that had been acclimated to 10° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		10	15	20	23	25	27
One fish	NN	--	--	--	--	130	4
	NS	773	413	360	215	130	6
	SN	--	--	--	--	135	4
	SS	770	407	340	155	125	12
Three fish	NN	--	--	--	--	100	< 5
	NS	877	472	517	297	152	16
	SN	--	--	--	--	67	7.5
	SS	960	480	382	225	95	15
Half of sample	NN	--	--	--	--	167	15
	NS	--	1,085	540	550	192	23
	SN	--	--	--	--	232.5	16.5
	SS	960	480	480	282.5	193.5	35.5
All of sample	NN	--	--	--	--	215	32.5
	NS	--	--	--	--	255	72.5
	SN	--	--	--	--	255	35
	SS	--	--	1,050	750	395	70

a Mean of replicated tests.
b NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

TABLE 4.—Mean values[a] of lethal exposure time (in minutes) for coho salmon that had been acclimated to 15° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		15	20	23	26	27	28
One fish	NN	--	--	--	--	26.5	8.5
	NS	420	265	2,325	222.5	38.5	7
	SN	--	--	--	255	23	10
	SS	70	36	45	29.5	17.5	12.5
Three fish	NN	--	--	--	--	50	10.5
	NS	430	295	297	245	51.5	14
	SN	--	--	--	705	30	14
	SS	175.5	78.5	62.5	38.5	32.5	18
Half of sample	NN	--	--	--	--	62	13.5
	NS	555	502.5	517.5	645	79	20.5
	SN	--	--	--	855	70	24.5
	SS	247	115	124.5	86	52.5	38
All of sample	NN	--	--	--	--	93	37
	NS	--	--	--	--	115	46
	SN	460	520	930	910	115	48
	SS	519	450	465	312.5	96	38

a Mean of replicated tests.
b NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

TABLE 5.—Mean values[a] of lethal exposure time (in minutes) for coho salmon that had been acclimated to 20° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		20	23	25	27	28	29
One fish	NN	--	--	--	93	35	14
	NS	375	558	400	130	29	11
	SN	130	--	15	47	17	7.5
	SS	89	20	135	45	13	9.5
Three fish	NN	--	--	--	104	47	14
	NS	497	510	510	152	38	13
	SN	--	--	--	82	34	13
	SS	180	300	165	69	30	12
Half of sample	NN	--	--	--	121.5	60	20
	NS	810	780	840	197	43	20
	SN	--	--	--	115	53.5	22
	SS	317.5	570	266	115	42.5	16
All of sample	NN	--	--	--	--	93.5	37
	NS	920	1,040	930	910	115	48
	SN	--	--	--	--	115	46
	SS	619	450	465	307	97	38.5

a Mean of replicated tests.
b NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

TABLE 6.—Mean values[a] of lethal exposure time (in minutes) for hatchery spring chinook salmon that had been acclimated to 15° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		15	20	23	25	27	28
One fish	NN	--	--	--	90.5	22	5
	NS	510	585	375	210	16.5	4.5
	SN	450	--	390	30	75	4
	SS	32.5	30	30	20.5	6.5	3
Three fish	NN	--	--	--	255	55	9
	NS	510	735	510	198	45	8.5
	SN	--	--	--	245	9	6.5
	SS	217	190	110	80	11	4
Half of sample	NN	--	--	--	360	66	115
	NS	675	820	540	255	52.5	10.5
	SN	--	--	--	370	39.5	9
	SS	315	165	123	122.5	16.5	6
All of sample	NN	--	--	--	1,020	88.5	30.5
	NS	900	1,080	755	275	97	25
	SN	--	--	--	700	78.5	19.5
	SS	720	780	525	399	50	40.5

a Mean of replicated tests.
b NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

TABLE 7.—Mean values[a] of lethal exposure time (in minutes) for hatchery spring chinook salmon that had been acclimated to 20° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		20	23	25	27	28	29
One fish	NN	--	--	720	34	33.5	5
	NS	255	150	--	71.5	38.5	9.5
	SN	--	--	41.5	6	8.5	5.5
	SS	30	30	7.5	15	10.5	3
Three fish	NN	--	--	--	84.5	44.5	6.5
	NS	360	345	372	86	43.5	14.5
	SN	--	--	795	39.5	16	7.5
	SS	45	67.5	30	28	16.5	4.5
Half of sample	NN	--	--	--	95	51.5	8
	NS	450	435	570	97	48.5	17
	SN	--	--	620	66	23	8.5
	SS	75	176	42.5	44	22	6.5
All of sample	NN	--	--	--	153.5	63.5	13.5
	NS	780	690	750	133	66	27.5
	SN	--	--	820	110	46.5	19
	SS	397	675	600	97.5	52.5	13.5

a Mean of replicated tests.
b NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

TABLE 8.—Mean values[a] of lethal exposure time (in minutes) for wild spring chinook salmon that had been acclimated to 10° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		10	15	20	23	25	27
One fish	NN	--	--	61	118	9	4
	NS	600	645	463	163	17	3
	SN	--	98	312	4	2	1
	SS	38	35	68	10	3	1
Three fish	NN	--	--	--	--	192.5	27
	NS	--	--	--	720	465	43
	SN	--	--	--	--	80	9
	SS	660	660	600	780	71.5	6
Half of sample	NN	--	--	--	--	82.5	5
	NS	--	--	780	405	195	4
	SN	--	--	--	1,080	7.5	2
	SS	495	187	150	58	7.5	3
All of sample	NN	--	--	--	--	193	27
	NS	--	--	--	900	165	43
	SN	--	--	--	--	80	9
	SS	870	660	600	780	71	6

a Mean of replicated tests.
b NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

TABLE 9.—Mean values[a] of lethal exposure time (in minutes) for steelhead trout that had been acclimated to 10° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		10	15	20	23	25	27
One fish	NN	--	--	930	458	60	4.5
	NS	195	172.5	230	230	60	8.5
	SN	--	360	--	10	6	6
	SS	57.5	15	6	6	8.5	5.5
Three fish	NN	--	--	--	--	200	10
	NS	280	217	367	320	112	15
	SN	--	--	--	--	206	9
	SS	76	50	71	52	19	11
Half of sample	NN	--	--	--	--	225	14
	NS	307	340	493	450	165	17
	SN	--	--	--	--	366	14
	SS	104	75	105	74	38	15
All of sample	NN	--	--	--	--	675	34
	NS	660	660	780	690	465	80
	SN	--	--	--	--	--	52.5
	SS	347.5	307.5	202.5	202.5	95	30

a Mean of replicated tests.
b NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

TABLE 10.—Mean values[a] of lethal exposure time (in minutes) for steelhead trout that had been acclimated to 15° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		15	20	23	26	27	28
One fish	NN	--	--	--	225	23	18
	NS	570	480	480	207	37.5	15
	SN	--	--	--	7	9.5	6
	SS	15	25	14	14	8	4.5
Three fish	NN	--	--	--	660	62.5	21
	NS	645	570	600	390	53.5	20
	SN	--	--	--	151.5	16.5	11.5
	SS	30	56	30	21.5	14	11
Half of sample	NN	--	--	--	--	75	24
	NS	840	630	645	435	58	23.5
	SN	--	--	--	495	23.5	15
	SS	45	94	52.5	43	19	19
All of sample	NN	--	--	--	--	107.5	42
	NS	1,320	870	900	720	81.5	34.5
	SN	--	--	--	840	127.5	32.5
	SS	690	452.5	157.5	225	57.2	24.5

a Mean of replicated tests.
b NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

stressed fish were subjected to temperature increases in water supersaturated at 125 to 130%, however, the prior stress significantly decreased their tolerance at temperatures below 26° C. Figure 1 shows only the data from populations acclimated to 15° C (Tables 3, 6, and 10), but similar curves that clearly show the effect of supersaturation can be constructed from the data on populations acclimated to the other temperatures.

Populations of coho salmon, steelhead trout, and chinook salmon acclimated to higher temperatures were able to tolerate higher temperatures for longer periods in supersaturated water as well as in normally saturated water. A comparison of our LE_{50} curves for coho with those of Brett (1952) indicates that tolerance to higher temperatures in supersaturated water

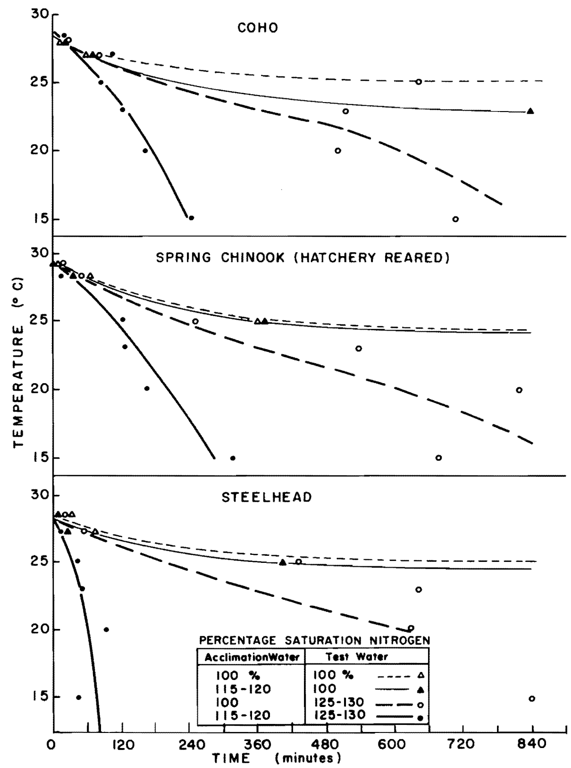

FIGURE 1.—Comparison of LE_{50} curves of hatchery-reared juvenile coho salmon, spring chinook salmon, and steelhead trout acclimated at 15° C and stressed at various levels of nitrogen saturation.

was considerably lower at exposures over 100 min for the same acclimation temperature than at normal saturation levels (Figure 2).

A comparison of the NN and SN curves (Figure 1) indicates that the prior stress of supersaturation of nitrogen had little effect on the fish when they were subjected to test water that was not supersaturated. This suggests that migrating salmon and trout under stress from supersaturation of nitrogen gas could recover from the effects of supersaturation if there were river areas where water would equilibrate. These data also show that salmon and trout populations acclimated at 15° C and subjected to nitrogen saturation of 125 to 130% will probably have about 50% mortality in less than 360 min with no temperature increase when stressed for 12 hr before testing and that subjecting the populations to temperature increases merely reduces the time to death.

In comparing the tolerance to temperature increases between coho and spring chinook salmon, we found that the results from the control tests—where both acclimation and test water were at 100% saturation—were similar to the results of Brett (1952). That is, coho were more tolerant than chinook and the respective upper lethal temperatures were 25 to 26° C. Brett did not study steelhead trout. We found that steelhead trout were nearly identical to coho in their tolerance

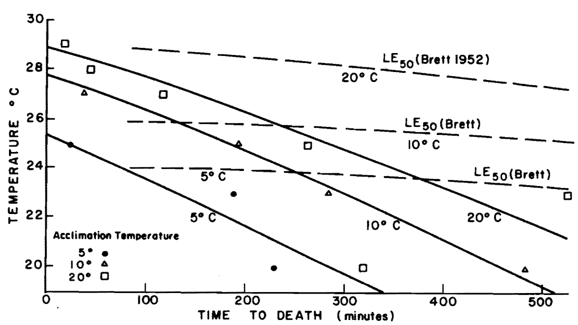

FIGURE 2.—Median-resistance-time (LE_{50}) plotted from temperature tolerance tests with coho salmon fingerlings acclimated at 5°, 10°, and 20° C and stressed for 12 hr at 115-120% saturation of N_2, then subjected to temperature increases in water supersaturated at 125-130%. Brett's LE_{50} curves for fish acclimated at 5° and 20° C—without N_2 stress—are shown for comparison.

to temperature increases when supersaturation of nitrogen was not present (control tests) but were the most vulnerable species when supersaturation was entered as a factor. Figure 3 compares the tolerance of three species to temperature increases when acclimation water and test water were supersaturated. Coho were the most tolerant, wild spring chinook next, and steelhead the least tolerant.

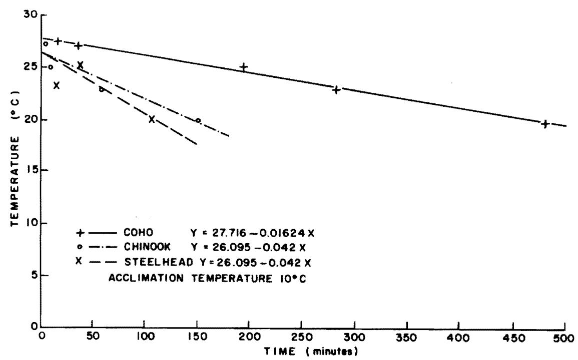

FIGURE 3.—Comparison of LE_{50} values between species of salmonid juveniles (acclimated at 10° C) in tolerance to temperature increases when stressed by 115-120% saturation of nitrogen gas for 12 hr, then subjected to temperature increases in supersaturated water at 125-130% nitrogen.

Size of fish at time of testing could influence the comparison between species. Coho and wild spring chinook acclimated at 10° C were nearly the same size at time of testing (Table 1), but steelhead acclimated at 10° C were larger than the other species when tested. There is evidence that extremely small chinook fry are more susceptible to nitrogen supersaturation than fingerlings; then, as the fingerlings increase in size, they become more susceptible than the small fingerlings but less susceptible than the fry (Meekin, 1969).[2] If this occurs with steelhead also, it could account for their lower tolerance. During one test with coho acclimated at 15° C, we found no differences in susceptibility within the size range tested (101-151 mm) at 125 to 130% saturation.

[2] Personal communication, Thomas Meekin, Washington State Department of Fisheries. Experiments at Priest Rapids Dam.

We intended to compare hatchery-reared chinook salmon with wild or naturally migrating spring chinook salmon acclimated to the same temperature. Wild fish were available only when they were acclimated to temperatures near 10° C. The hatchery population had deteriorated by then, however, so we compared wild fish acclimated at 10° C and hatchery fish acclimated to 15° C (Figure 4). As expected, the hatchery population—acclimated at the higher temperature—was able to tolerate the highest temperatures for a longer period, but when the LE_{50} curves—which include the effect of nitrogen—are compared, little difference can be noted. This indicates that results achieved in the laboratory with hatchery stocks can be applied to wild Columbia River stocks with reasonable accuracy.

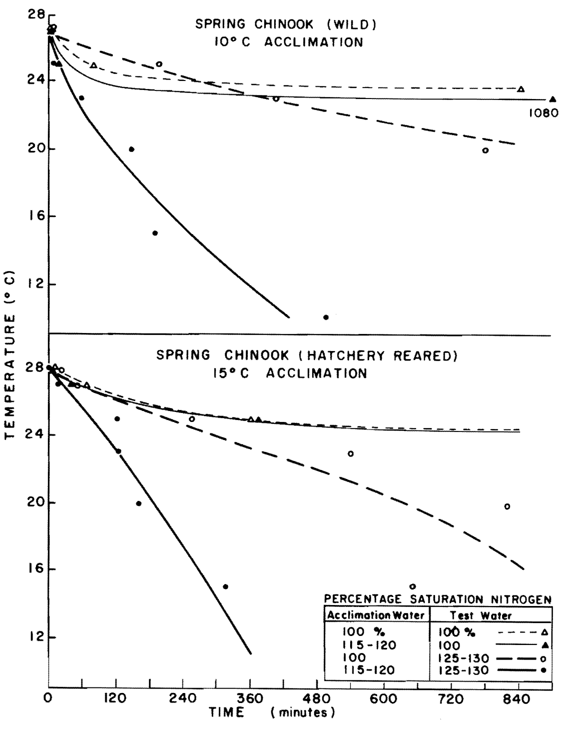

FIGURE 4.—Comparison of LE_{50} curves between juvenile wild and hatchery spring chinook salmon at various temperatures and levels of saturation of nitrogen gas.

EFFECT OF DEPTH ON RELATION BETWEEN SUPERSATURATION OF NITROGEN AND TOLERANCE OF JUVENILE FISH TO TEMPERATURE INCREASES

Examination of fish in cages at the forebay of Priest Rapids Dam (Ebel, 1969) indicated that juvenile coho and chinook salmon would not contract gas bubble disease if held at a sufficient compensating depth (5 m). This finding suggests that fish subjected to temperature increases in addition to nitrogen supersaturation would also be less affected if they remained at sufficient depth when they encountered a temperature increase.

To test this hypothesis, we subjected coho salmon acclimated at 10° C to three temperatures above acclimation in water supersaturated at 130% in the 9-m (deep) tank where they could select any depth from the surface to 9 m; we then compared LE_{100} curves in the 20-cm (shallow) tanks with those in the deep tank (Figure 5). These curves definitely indicate that the coho benefited by having the option to sound in the deep tank. The LE_{100} level never was reached during the 18-hr observation period when the fish were subjected to 20° C (10° C increase) in the deep tank, but occurred after

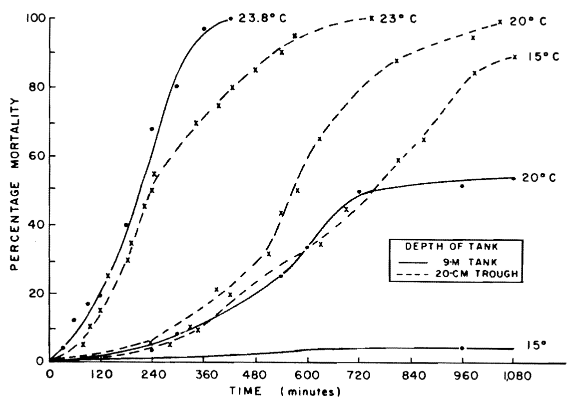

FIGURE 5.—Comparison of LE_{100} curves for coho salmon acclimated at 10° C and subjected to three temperatures (15°, 20°, and 23° C) in 20-cm and 9-m deep tanks containing water supersaturated with nitrogen gas at 130% saturation. Oxygen concentrations varied from 115 to 125% saturation.

about 17 hr in the shallow tank. Similarly at 15° C (5° C increase), the LE_{50} level was never reached in the deep tank but was reached in about 12.5 hr in the shallow tank. No benefit from depth is indicated in the curves at 23° C; in this comparison, the fact that temperature in the deep tank was 0.8° C higher (23.8° C) than that in the shallow tank could account for the lack of difference.

Wild juvenile spring chinook salmon from the gatewells at McNary Dam also were tested in the deep and shallow tanks. These fish were acclimated at 10° C and then subjected to a 5° C increase (15° C) with supersaturation of nitrogen gas at 130% saturation. The fish also were stressed for 12 hr before the test in 10° C water supersaturated at 120% saturation. Again, chinook tested in the deep tank survived at a higher rate than those in the shallow tanks; the LE_{50} was never reached in the deep tank, whereas 100% mortality was reached in approximately 11 hr in the shallow tanks (Figure 6).

Observations in the deep tank during tests with the coho and chinook salmon indicated that most fish remained between about 1 and 4 m of the surface. Light intensity and turbidity possibly influenced the depth distribution. During these tests, artificial light at an intensity of about 100 footcandles was present at the surface of the water. Turbidity in the tank was minimal; a Secchi disc was visible at the bottom of the tank and the Jackson turbidity unit measurement was 0.

It is difficult to relate tests in the tank to natural conditions because turbidity in natural water varies greatly. In the Snake River, turbidity as measured by a Secchi disc varies from 0.2 to 8.0 m, depending on season and location. Turbidity usually is high during the spring runoff in both the Snake and Columbia Rivers; readings are seldom over 1 m on the Snake River (Ebel and Koski, 1968). This high turbidity limits visible light penetration to a maximum of about 1.5 m (observation verified by scuba diving). We therefore believe that juveniles as observed in the tank were at greater depths than they might be in the Snake or Columbia Rivers during the spring migration. Durkin, Park, and Raleigh (1970) found that most juvenile salmon were near the surface as they entered Brownlee Reservoir. Fish in the Columbia and Snake Rivers apparently do not sound to a depth sufficient to compensate for nitrogen saturation levels exceeding 130%; hence the mortalities reported herein are probably on the conservative side. We also emphasize that even though the option of having sufficient depth reduced the mortality rate, substantial mortalities occurred.

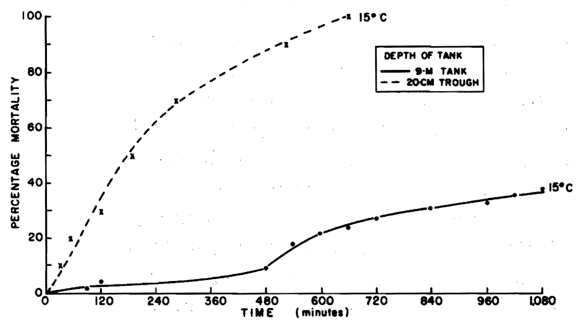

FIGURE 6.—Comparison of LE_{100} curves of wild spring chinook salmon acclimated at 10° C and subjected to a 5° C increase (15° C) in tanks 20 cm and 9 m deep that were supersaturated with nitrogen gas at 130% saturation. Oxygen concentrations varied from 115 to 125% saturation.

TEMPERATURE STANDARDS FOR RIVERS WITH NITROGEN SUPERSATURATION

Our test temperatures and experimental design were purposely selected so that these data could be compared with the results reported by Brett (1952). Brett cautions that the information he presents should not be applied verbatim to other environments. Because of the excellence of his work and the lack of later findings concerning temperature tolerance of Pacific salmon, the upper lethal levels established in his paper are widely quoted and used for setting temperature tolerance standards for rivers and streams containing salmon—without regard to other physical and chemical characteristics of the water. The changes in Brett's tolerance curves caused by the stresses of supersaturation of nitrogen gas were obvious.

Although complete statistical analysis of our data are not presented in this paper, the differences shown between tolerance curves of fish tested in water with and without supersaturation of nitrogen are so great that conclusions concerning the effect of supersaturation can be made with relative confidence.

Substantial mortalities will occur to migrating juvenile salmon and trout in the Columbia and Snake Rivers—even if no thermal plume or increase in temperature is encountered—whenever the populations must pass through large areas where 125 to 130% saturation of nitrogen occurs. Studies of vertical distribution (e.g., Smith, Pugh, and Monan, 1968; Durkin et al., 1970) indicate that the majority of migrants are in surface waters, with substantial numbers in waters less than 2 m deep. This is too shallow to compensate for nitrogen levels as high as 130%. Surveys of nitrogen levels by Ebel (1969), by Beiningen and Ebel (1971), and by NMFS and State fisheries personnel of Washington and Oregon during the 1970 spring migration, verify that nitrogen in large areas of both rivers exceed 130% saturation. Examination of fish in cages suspended on the surface and at various depths revealed that mortalities caused by nitrogen often exceeded 40% in a deep (4.5 m) cage where the fish could sound at their volition. Periodic checks of juveniles in the Snake River by NMFS personnel in 1970 indicated that 25 to 45% of the chinook salmon and 30 to 58% of the steelhead trout migrants arriving at Ice Harbor Dam had external symptoms of gas bubble disease. We made similar observations of migrants at The Dalles and McNary Dams in 1968 and 1969 and recorded similar findings.

Obviously the migrating juvenile salmon and trout in the Columbia and Snake Rivers are under stress during periods of nitrogen supersaturation. Any increase in temperature over the ambient river temperature, then, will harm these populations. Mortalities already occurring will be accelerated even with minimal temperature increases. Our data show that LE_{50} levels of temperature (Figures 1-4) are far higher than could be accepted as standards for upper limits of rivers containing trout and salmon even at normal concentrations of dissolved nitrogen. The time to first mortality of wild spring chinook salmon, for example, that were acclimated to 10° C and tested in supersaturated water at 23° and 25° C was 10 and 3 min, respectively (Table 8). Temperatures and temperature increases such as these occur in thermal effluents (Coutant, 1969), and substantial mortalities could occur to juvenile salmon and trout passing through thermal plumes.

During spring and summer when flows are low, increases in temperature of the Columbia River from Priest Rapids Dam to the forebay of McNary Dam have been as high as 2.5° C (Ebel, 1969). Increases in temperature over the acclimated temperature greatly accelerated time to death of juveniles when supersaturation of nitrogen gas was present in the test water whether the fish were held in shallow or deep tanks. However, during the low flow periods when temperature increases such as this occur, nitrogen saturation levels are usually low and mortalities such as indicated in the tests would not occur.

The obvious results of these tests are that supersaturation of nitrogen must be considered when setting temperature standards and that any increase allowed over the ambient temperature of the river during periods when the river is supersaturated with nitrogen will be detrimental to salmon and trout populations.

CONCLUSIONS

1. Supersaturation of nitrogen drastically affects the tolerance of juvenile coho salmon, chinook salmon, and steelhead trout to temperature increases. Tolerance to increases below 26° C is lowered and mortality rates are accelerated.

2. Acclimation to higher temperatures will enable the three species to tolerate higher temperatures longer when nitrogen supersaturation is a factor; however, 50% mortality will be reached in less than 18 hr at all acclimation temperatures with supersaturation of nitrogen at 125 to 130%. No temperature is suitable at the 125 to 130% level of nitrogen supersaturation.

3. Depth is an important compensating factor

when supersaturation of nitrogen is present. Tests in the deep (9-m) tank, where fish were free to roam from the surface to the bottom, revealed that mortality rates were much lower and tolerance to temperature increases was increased if the juveniles had the option to sound when subjected to temperature increases.

4. Coho were the most tolerant, chinook next, and steelhead the least tolerant to temperature increases when the water was supersaturated with nitrogen. When supersaturation was not a factor, coho and steelhead were about equally tolerant to temperature increases and chinook the least tolerant.

5. Any increase in temperature allowed over the ambient temperature (whether high or low) of the river during periods of supersaturation of nitrogen will be detrimental to migrating juvenile salmon and trout. Temperature standards should account for the effect of supersaturation of nitrogen gas.

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