FINAL REPORT

ON

AN EXPLORATORY STUDY OF INTERACTION OF INCREASED TEMPERATURE AND NITROGEN SUPERSATURATION ON MORTALITY OF ADULT SALMONIDS

TO

U.S. BUREAU OF COMMERCIAL FISHERIES

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CHARLES C. COUTANT

WITH

ROBERT G. GENOWAY

BATTELLE MEMORIAL INSTITUTE

PACIFIC NORTHWEST LABORATORY

RICHLAND, WASHINGTON

FORWARD

This report summarizes research conducted by Battelle-Northwest (Battelle Memorial Institute/Pacific Northwest Laboratory) for the U. S. Bureau of Commercial Fisheries, Seattle Biological Laboratory, under Contract Number 12-17-0001-1785 during the period 3 January through 28 November 1968. Experiments were conducted in July 1968 in the Aquatic Biology facility of the U. S. Atomic Energy Commission (AEC), Richland, Washington, as provided in Contract AT(45-1)-1831 between Battelle-Northwest and the AEC. The work was under the direction of Dr. C. C. Coutant. The experiments were carried out by Mr. R. G. Genoway. Additional technical assistance was provided by Mr. E. G. Tangen and Mr. C. O'Malley. Chinook salmon were provided by Mr. W. J. Ebel, U. S. Bureau of Commercial Fisheries. Dissolved nitrogen analyses were carried out under the direction of Dr. B. W. Mercer.

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INTRODUCTION

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of supersaturation of dissolved nitrogen in the Columbia River between Grand Coulee Dam and the estuary (W. J. Ebel, unpublished). 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 (Gorham 1898, 1899; Marsh 1903, 1904; Marsh and Gorham 1905; Plehn 1924; Haempel 1928; Mrsic 1929; Wiebe and McGavock 1932; Wiebe 1933; Schaeperclaus 1933; Woodbury 1941; Engelhorn 1943; Alikunhi et al. 1951; Matsue et al. 1953; Rucker and Hodgeboom 1953; Schaperclaus 1954; Ruckavina and Varenika 1956; Doudoroff 1957; Egusa 1959; Bishai 1960; Harvey and Smith 1961; Harvey and Cooper 1962; Renfro 1963; Westgard 1964; Shirahata 1966; Pauley and Nakatani 1967). 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 (Rukavina and Varenika 1956, Doudoroff 1957). A drop in hydrostatic pressure induces bubble formation in the fish tissues.

Symptoms have been recognized both in adults and in young. 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 and often frantic swimming is followed by a period of quiet before death. Very small fish may 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 used by him to create test situations. In these, 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 temperatures during migration (Ebel, personal communication). Abrupt differences between external and internal gas tensions may be created as fish enter tributary streams, change depths at fish passage

facilities at dams or pass through mixing zones below thermal discharges. 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 objective of this exploratory study has been to determine whether a 5° C water temperature elevation that is not in itself lethal to chinook salmon as determined by laboratory studies with immature fish (i.e., from 16° C up to 21° C) will result in mortalities when combined with effects of supersaturated gasses. Two conditions of gas interaction were considered, namely 1) acclimation to supersaturation at the lower temperature prior to temperature rise, and 2) supersaturated water during exposure to the elevated temperature.

SUMMARY AND CONCLUSIONS

Water temperature of about 22° C is lethal to adult chinook salmon within 24 hours when accompanied by nitrogen saturation values exceeding about 115%. This contrasts with a 24-hour lethal level in the literature of above 25° C for temperature alone, and significantly longer survival in these experiments when temperature was acting alone.

Gas acclimation condition has little practical bearing on survival at 22° C under high gas saturations although accumulated chronic effects of high gas acclimation cause these fish to die somewhat faster than low gas acclimated fish. Evident distress of all fish and rapid mortality of some individuals results when fish acclimated to high gas saturations increase in temperature by about 5° C while entering water of lower

saturation value. External gas bubble symptoms that are evident on fish exposed to chronic high gas saturation disappear rapidly following death, nearly all being lost within 24 hours.

EXPERIMENTAL PROCEDURES

Design

Fish were held in two gas conditions for acclimation purposes prior to testing, one with high gas saturation values (> 118%) and one with reduced saturation values (< 110%). No attempt was made to determine whether the fish were truly acclimated to the different gas levels prior to testing. One week was selected as the "acclimation" time. Four conditions of transfer were made, with 5 fish having each acclimation history being placed in low and high gas saturation test water that was approximately 5° C higher than the acclimation temperature. It was planned that control fish from each acclimation condition would be handled similarly to test fish, then retained in each acclimation tank during experiments. A total of 30 fish, including controls, would have been involved in a test. Mortality of high gas acclimated fish reduced the numbers of these fish available for controls, however. Observations were made of the physical condition of all fish for about 10 days following transfers

Water Supply and Holding Tanks

Test water was obtained by pumping from the Columbia River. During the experimental period (29 April Through 9 May and 4 through 25 July), the Columbia River was at elevated river flows and the water was super-saturated with nitrogen (Table 1). The elevated gas saturations persisted

TABLE 1. DISSOLVED NITROGEN ANALYSES

Station	Sampling date	Water Temp. °C	Bar. Press.[1] mmHg	Dissolved[2] N_2 mg/l	Percent Saturation N_2
Treated tank[3]	4-16	8.9	743	19.7	104
Pressurized hatchery supply	4-19	8.6	749	21.8	115
Treated tank	4-19	8.8	749	20.7	108
Treated tank	4-30	12.5	742	17.5	106
River at intake	4-30	12.2	742	20.1	122
Treated tank	4-30	17.5	742	16.9	96
Untreated tank	4-30	17.2	742	20.6	116
Untreated tank	6-27	15.7	760	20.4	118
Treated tank	6-27	16.0	760	15.7	90
River at intake	6-27	15.6	760	19.1	110
Treated tank	7-3	16.5	760	18.1	107
Untreated tank	7-3	16.4	760	20.8	124
Untreated tank	7-8	18.3	757	20.5	125
Treated tank[4]	7-8	18.6	757	16.8	103
Untreated test tank	7-8	22.1	757	20.1	129
Treated test tank	7-8	22.2	757	16.0	103
Untreated test tank	7-11	21.8	761	22.3	134
Treated test tank	7-11	22.0	761	18.3	110

[1] Barometric pressure at Aquatic Laboratory used to compute saturation values.

[2] Precision of measurements at 95% confidence level calculated to be ± 3.6%.

[3] Treated for removal of supersaturated nitrogen.

[4] Test tanks heated about + 5° C over hatchery supply temperature.

in the experimental tanks when the water was delivered through high pressure lines directly from the river pump.

The pressure at the pump generally varies between 100 and 120 psi while in the vicinity of the fish tanks this pressure has dropped to 60 to 90 psi. Water pressure was further reduced to 15 to 20 psi in a spring-loaded pressure regulating valve or in a small head tank before the water passed through a flowmeter and into the tank. To reduce air equilibration of gasses, the tank inlet was below the water surface.

Low gas saturation values were created by foaming the supply water with compressed air. The device for this air equilibration is shown in Figure 1. Three lengths of 34-inch long, 3/4-inch diameter, porous polyethylene tubing delivered a barrage of bubbles to a 3-inch deep water layer over them in a 4' x 15" x 15" fiberglass hatchery trough. Air pressure was maintained at about 15 psi. Water moving the length of the trough at a rate of about 4 U.S. gallons per minute was reduced in gas content to essentially air equilibrium. From the degassing trough, water was introduced directly to the fish tank with the inlet above the water surface.

Temperature control was not exerted on water to acclimation tanks, rather the tanks followed daily and seasonal changes in hatchery water supply temperature. These temperatures, which were close to river water values, are summarized in Table 2. Periods of fish holding are indicated.

TABLE 2. TEMPERATURE OF HATCHERY SUPPLY WATER (°C), AND PERIODS OF ACCLIMATION AND TESTING

		MAX	MIN	ACCL.	TEST 1	TEST 2
April	29	13.3	12.5	*		
	30	12.2	11.7	*		
	1	12.4	11.2	*		
	2	12.7	11.6		*	
	3	13.2	11.7		*	
	4	13.4	12.2		*	
	5	13.4	12.6		*	
	6	13.4	12.8		*	
	7	13.2	12.1		*	
	8	13.2	12.0		*	
	9	13.8	12.8		*	
June	25	16.1	15.1	x		
	26	16.1	15.5	x		
	27	16.0	15.3	x		
	28	15.6	15.1	x		
	29	15.6	15.0	x		
	30	15.7	14.8	x		
	1	16.0	15.0	x		
	2	16.1	15.1	x		
	3	16.5	15.3	x		
	4	16.9	15.7	x	x	
	5	17.4	16.3	x[(a)]	x	x
	6	17.7	16.8	x	x	x
	7	17.9	17.0	x	x	x
	8	18.4	17.2	x	x	x
	9	18.3	17.7	x	x	x
	10	18.3	17.5	x	x	x
	11	18.0	17.2	x	x	x
	12	17.8	17.2	x	x	x
	13	17.8	16.9	x	x	x
	14	17.5	16.7	x	x	x
	15	17.5	16.7			

(a) Only low gas acclimations continued past this date.

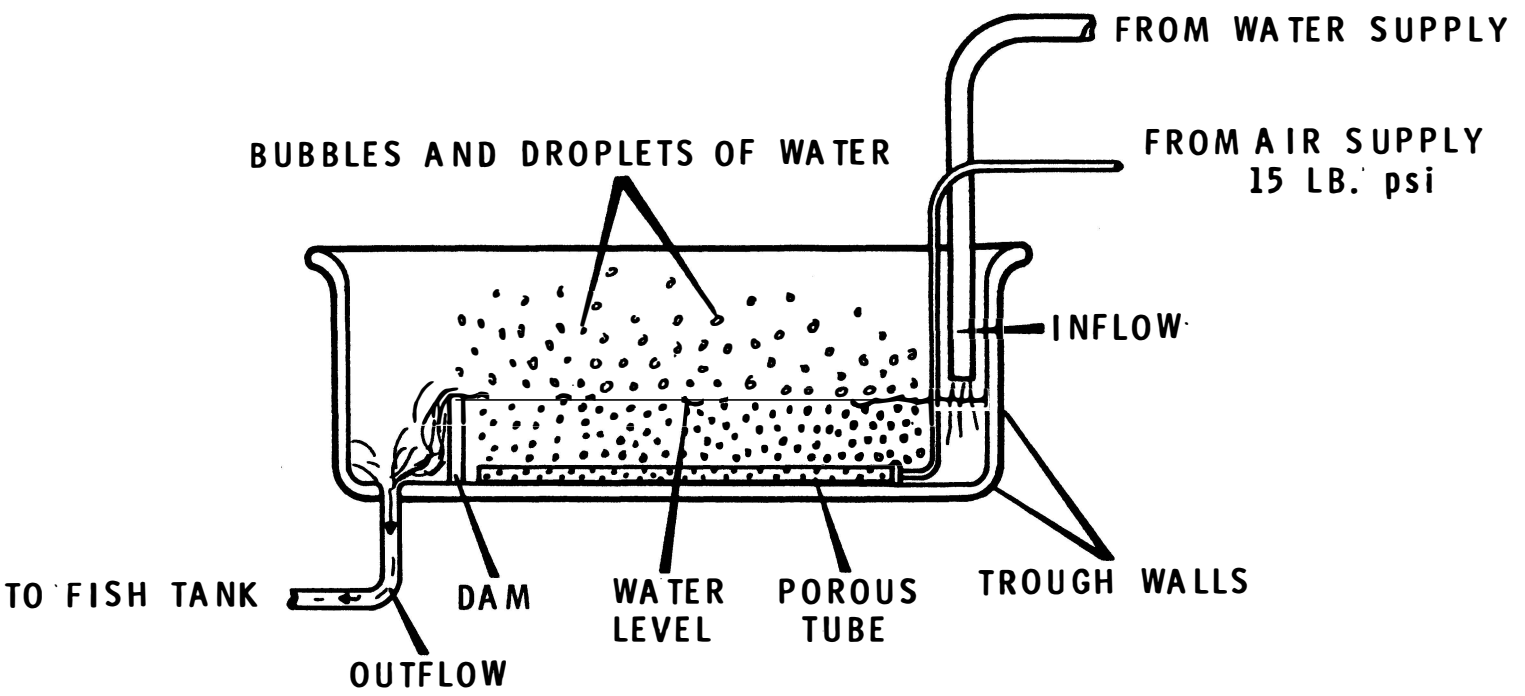

Fig. 1. Gas scrubber used for air equilibration of dissolved gasses in water.

Test water requiring 5° C temperature elevation over that of the hatchery supply was heated in a thermostatically controlled steam heat exchanger prior to entry into fish tanks and degasser. To compensate for some heat loss in the degassing system, water to the degasser was passed through a 1000-watt calrod heating unit controlled by a rheostat. In the chinook experiment, the elevated temperature at which the test was initiated was maintained throughout the period of fish observation. During rainbow tests, both acclimation and test tanks followed river temperature increases over the course of the test.

Fish holding tanks for gas acclimation were of non-insulated fiberglass construction, 6 feet in diameter, with an effective water depth of 30 inches. Calculated volume is 529 U. S. gallons or 2010 liters. Water was introduced by jetting either above (for degassed water) or below (for high gas saturations) the water surface causing a circular movement of water.

Test tanks were also fiberglass, but were 4 ft. in diameter with an effective water depth of 2 ft. Water circulation was similar to that in acclimation tanks.

Test Fish

A preliminary experiment to test the facilities was conducted using adult rainbow trout. These trout were 3-7 years old and weighed 1-4 kg. The original stock came from Natches hatchery. Rainbows used were from the brood stock maintained through several generations at the aquatic

biology facility at Hanford. The fish were transferred by dip net from the brood stock pond to the acclimation tanks used in this study.

The objective of this study was to investigate the responses of adult chinook salmon. Mature adults, however, are very large, difficult to use experimentally, and difficult to hold under enclosed conditions. Furthermore, mature adults are a premium resource. For these reasons, the present study was designed to use "jack" salmon, that is, precocious males that have undergone premature migration. These fish are quite abundant, they are smaller than mature adults, and they apparently serve little useful purpose in perpetuating the species when larger males are available.

Chinook jacks from the late spring run were collected for this study by personnel of the U. S. Bureau of Commercial Fisheries at the fish passage facility of McNary Dam. Fish entered an enclosure of their own free will at the upstream end of the Oregon fish ladder, from which they were transferred to a refrigerated fish transport truck. These fish were received at the aquatic biology facility of Battelle-Northwest over a four-day period, 25-28 June 1968. Half of each load of fish went directly to each of the two acclimation tanks. Table 3 summarizes the placement and initial mortalities of the fish supplied. Fish were 50-60 cm in length (snout to hypural plate) and weighed between 2 and 3 kg.

TABLE 3. PLACEMENT AND PRETEST MORTALITY OF JACK CHINOOKS.

	Number Received		Mortalities	
Date	High Gas Tank	Low Gas Tank	High Gas Tank	Low Gas Tank
6-25	7	6	0	0
6-26	6	7	0	0
6-27	9	9	0	0
6-28	11	7	2	0
7-2[1]	--		2	0
7-3	--[2]	-	1[2]	0
7-4	--	-[2]	6	0[2]
7-5	--	-	3	0
	33	29	14	0

[1]Date of tagging.

[2]Routine treatment of ponds with malachite green (0.75 ppm).

Fish were marked using Peterson tags (rainbow trout) and colored-wire spaghetti tags (chinook). Rainbow were caught individually, anesthesized in methylpentynol at a concentration of 1:200, and tagged while resting in a hanging canvas cradle. Chinook were lightly anesthesized in the acclimation tanks with Quinaldine at a concentration of 1:140,000, then placed individually in methylpentynol (1:200) in a 30-gallon plastic garbage can for further narcotization. Marking was done in a hanging canvas cradle, as with rainbows. Vinyl coated copper wire tags were inserted through the dorsal musculature posterior to the dorsal fin by means of a #13 hypodermic needle and tied in a square knot. Contrasting colors differentiated the two acclimation conditions.

RESULTS

Rainbow Trout

One 9-day test was conducted on rainbow trout principally for the purpose of testing the mechanics of the experiment. Acclimation and test temperatures at the start were 11.0 and 16.0° C, respectively. A full compliment of 20 test fish was used.

No mortalities were observed in test fish, although a control fish died acclimating to high gas conditions. Test fish from both gas acclimation levels showed lethargy in high gas test water. One fish acclimated and tested in high gas water developed bulging eyes and was apparently blinded. This condition was observed on the last day of examination, 8 days after the start of the test.

Chinook Salmon Acclimation

Fish acclimating in high gas saturations developed some externally-visible symptoms of gas-bubble disease within 24 hours. These were small bubbles in dorsal fins. Coloration and alertness were affected after four days, at which time fungus infection was apparent on some affected areas. Figures 2 through 7 illustrate external bubble symptoms at day 5. Fish acclimating to low gas saturation values showed no disease symptoms, retaining good coloration, skin texture and alertness throughout the 27-day duration of testing.

Acclimation time prior to testing was reduced from the planned minimum of 7 days to 6, as fish losses rose on the 6th day in the high gas acclimation tank (Table 3). General condition of surviving fish in high gas water was poorer than in the low gas acclimation at the start of experimentation. Water temperatures during acclimation were well within accepted levels for salmon, however (Table 2).

Testing

The experiment with jack chinook was conducted with one replication. The first trial used a full compliment of 20 fish, with acclimation fish remaining as controls during the first day. The second trial used some fish acclimated as controls for the first experiment. All remaining fish acclimated to high gas conditions were used for testing in the second experiment, due to unexpectedly high mortalities. Acclimation and test temperatures at the start of trial number 1 were 16.0 and 21.5° C, respectively. At start of trial number 2, one day later, acclimation temperature was 17.0° C while the test temperature was maintained at 21.5° C.

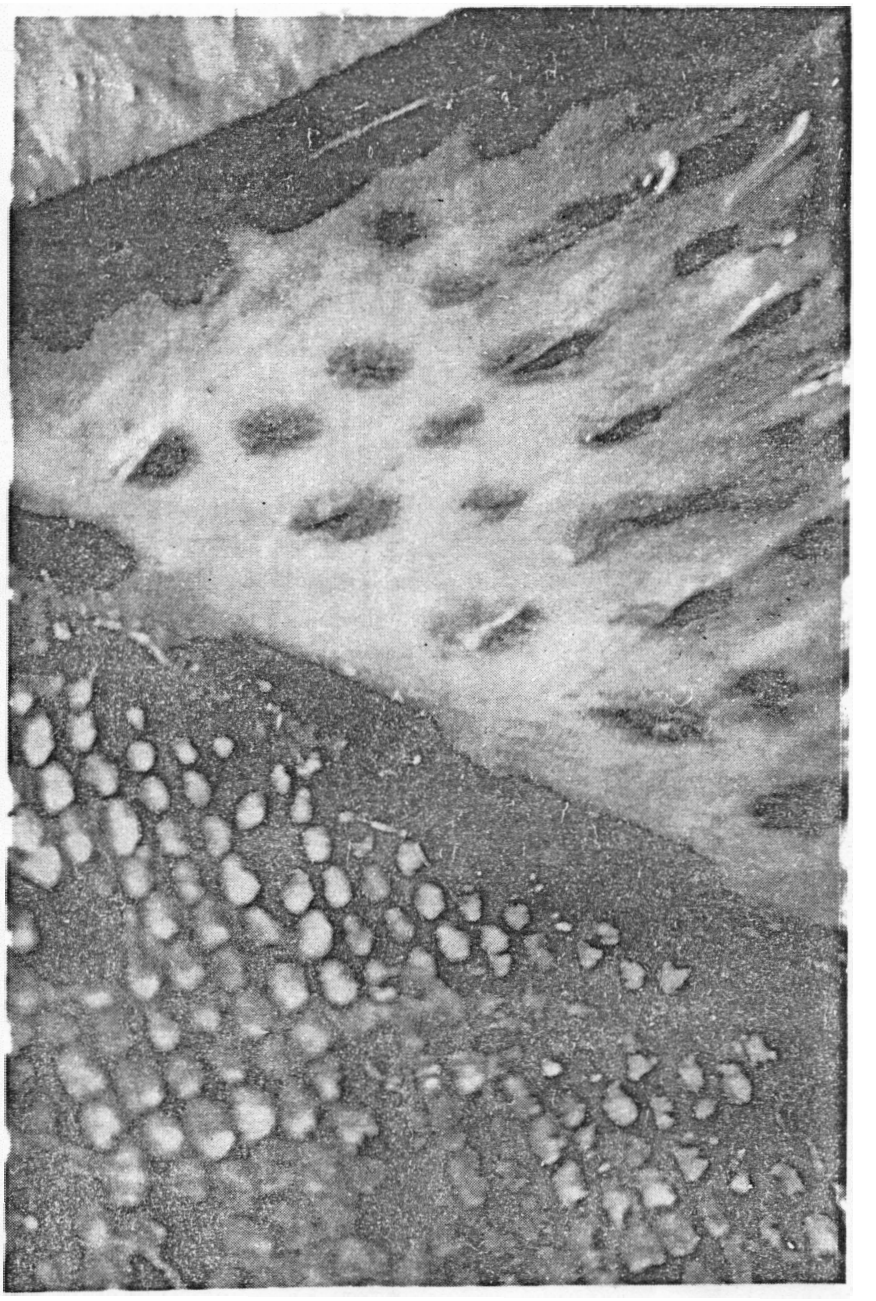

Fig. 2. Normal dorsal fin of salmon held 5 days in water with low gas saturation.

Fig. 3. Blistered dorsal fin and skin of salmon held 5 days in water with high gas saturation.

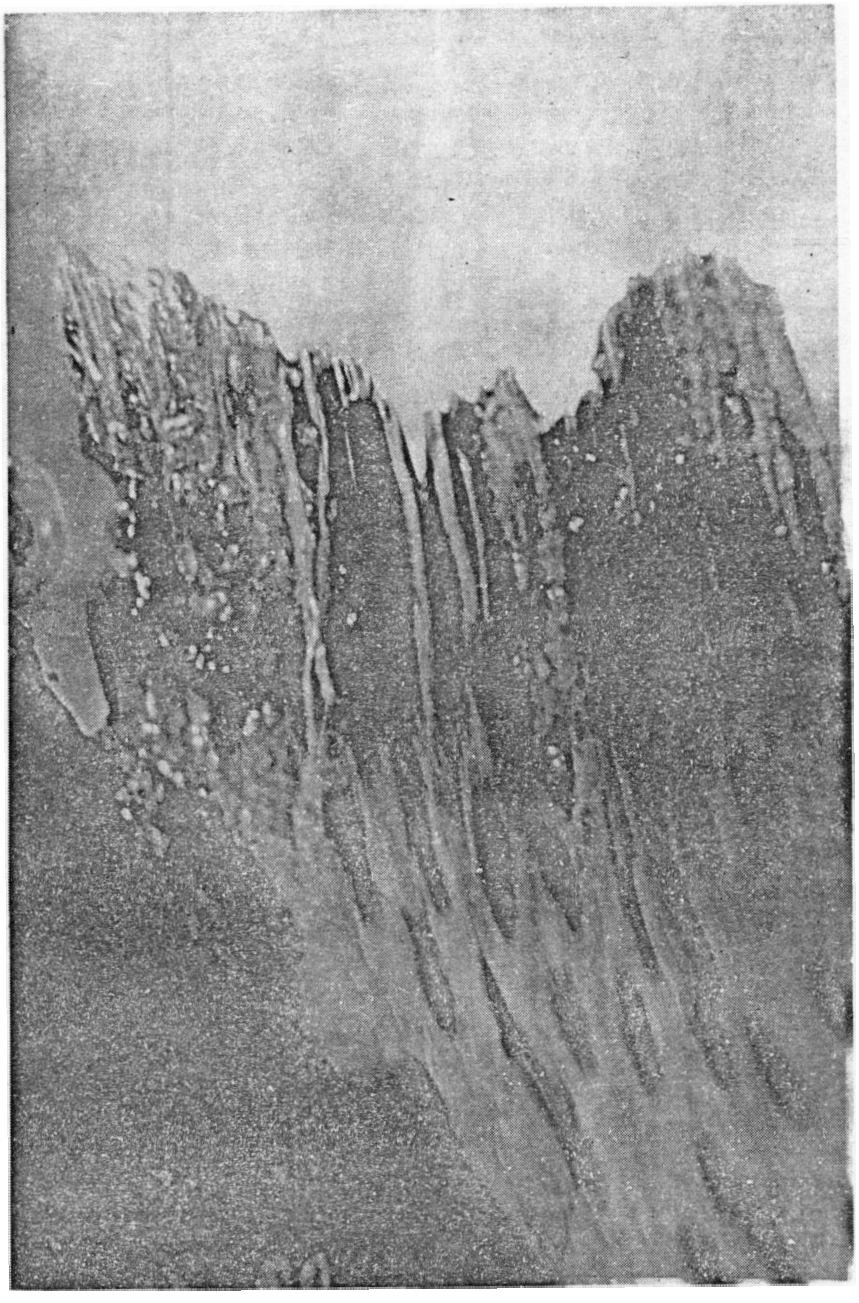

Fig. 4 Blistered dorsal fin of salmon held 5 days in water with high gas saturation.

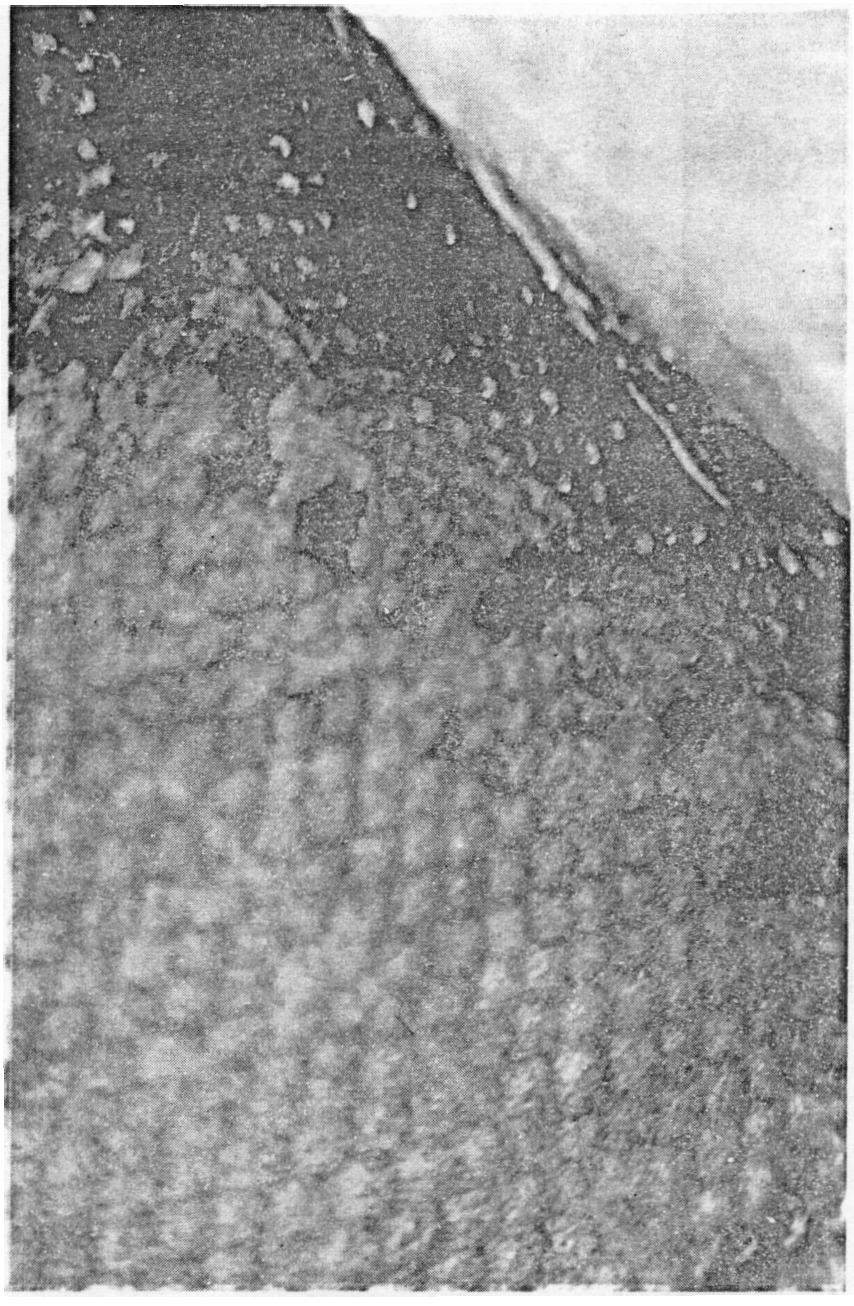

Fig. 5. Normal skin of salmon held 5 days in water with low gas saturation.

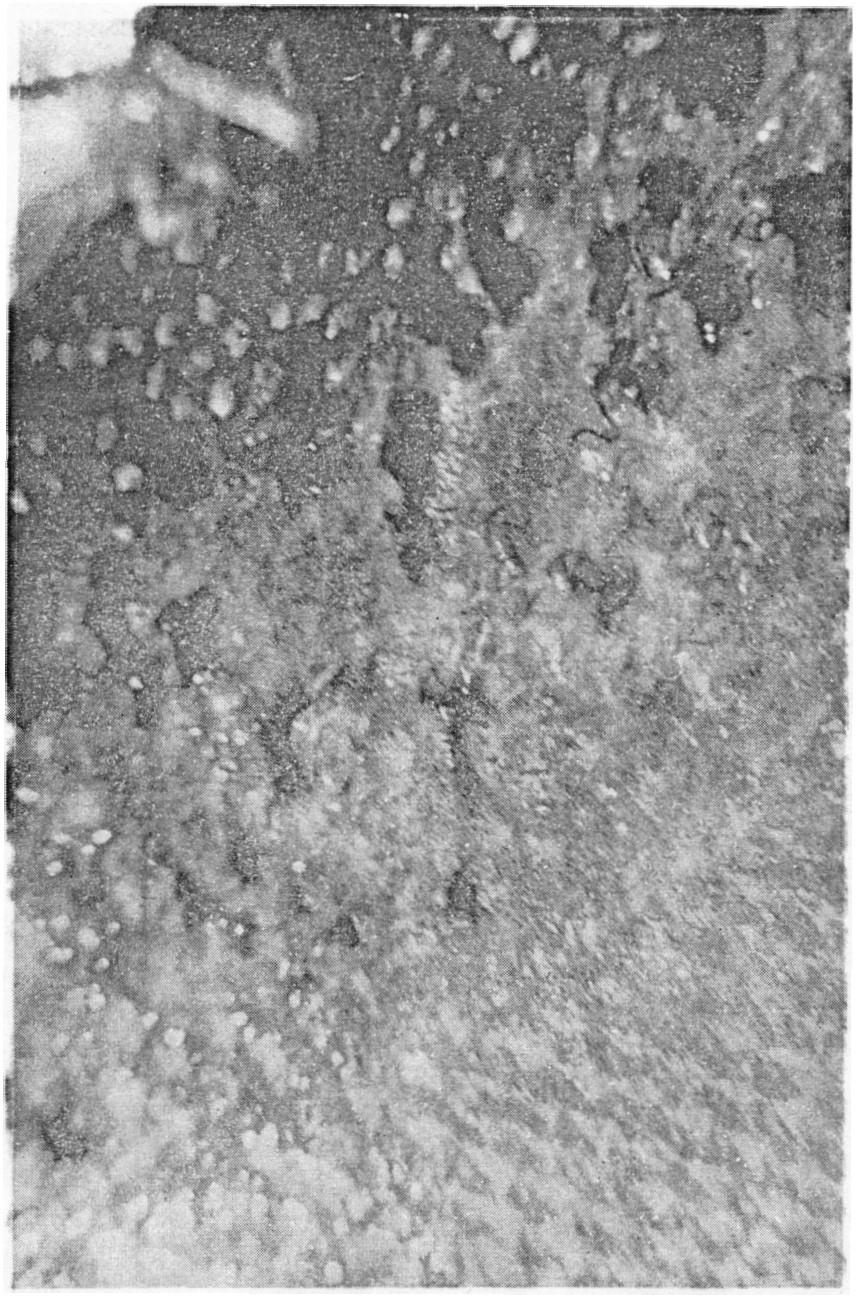

Fig.6. Blistered skin of salmon held 5 days in water with high gas saturation.

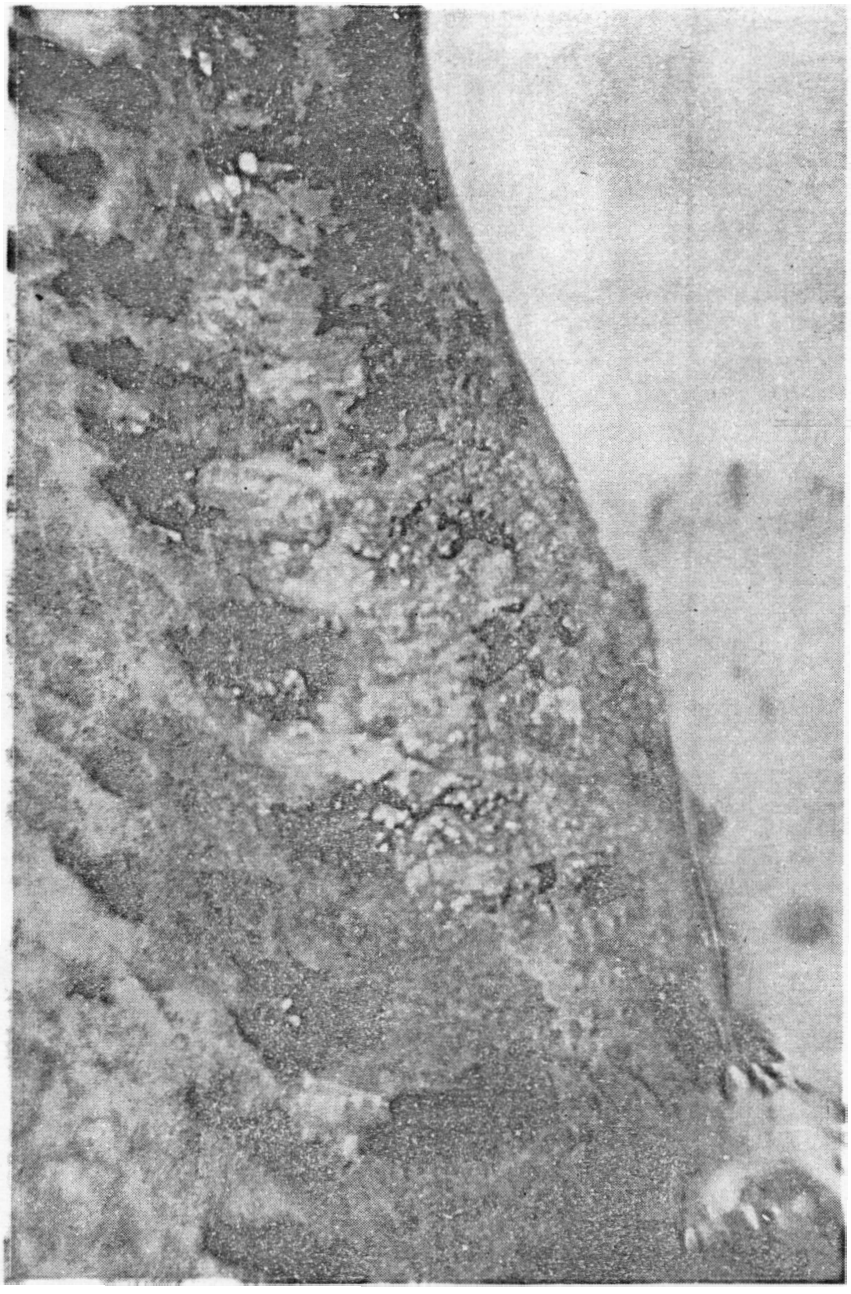

Fig. 7. Blistered skin of salmon held 5 days in water with high gas saturation.

The results of these two trials are presented as cumulative mortality histograms in Figure 8. The scale of the abscissa is time after transfer of fish to the test tank. The ordinate for each test condition is the cumulative number of deaths.

Most rapid mortality was seen in some high gas acclimated fish when they were placed into low gas, elevated-temperature water (B). All fish with this treatment showed signs of immediate stress, but most recovered from this initial ill effect. The rapid mortalities, two in one replicate and one in the other, occurred within one hour of the start of the test.

All fish in the high gas, elevated-temperature test treatments, regardless of gas acclimation condition (A, C), died within 24 hours. External blister symptoms were highly variable. Those fish acclimated to high gas saturations (A) died much sooner, with one exception, than did the fish acclimated to low gas. As a group, they died most rapidly of all.

Fish in low gas treatments (B, D) died gradually over a 10-day period. Several deaths, in addition to the very rapid mortalities noted above, occurred within the first day. This was observed even in the treatment (D) where high gas saturations were not involved either in acclimation or testing. No mortalities were observed during this period in fish retained in the low gas - low temperature acclimation tank.

Loss of Gas Blisters Following Death

Because of the obvious significance of diagnosis of gas bubble disease in the field following fish kills of questionable origin, the appearance of symptoms was followed in carcasses after death. Most external gas blisters were lost by dead fish in 17° C river water within 24 hours. Small bubbles in fins and under scales disappeared in

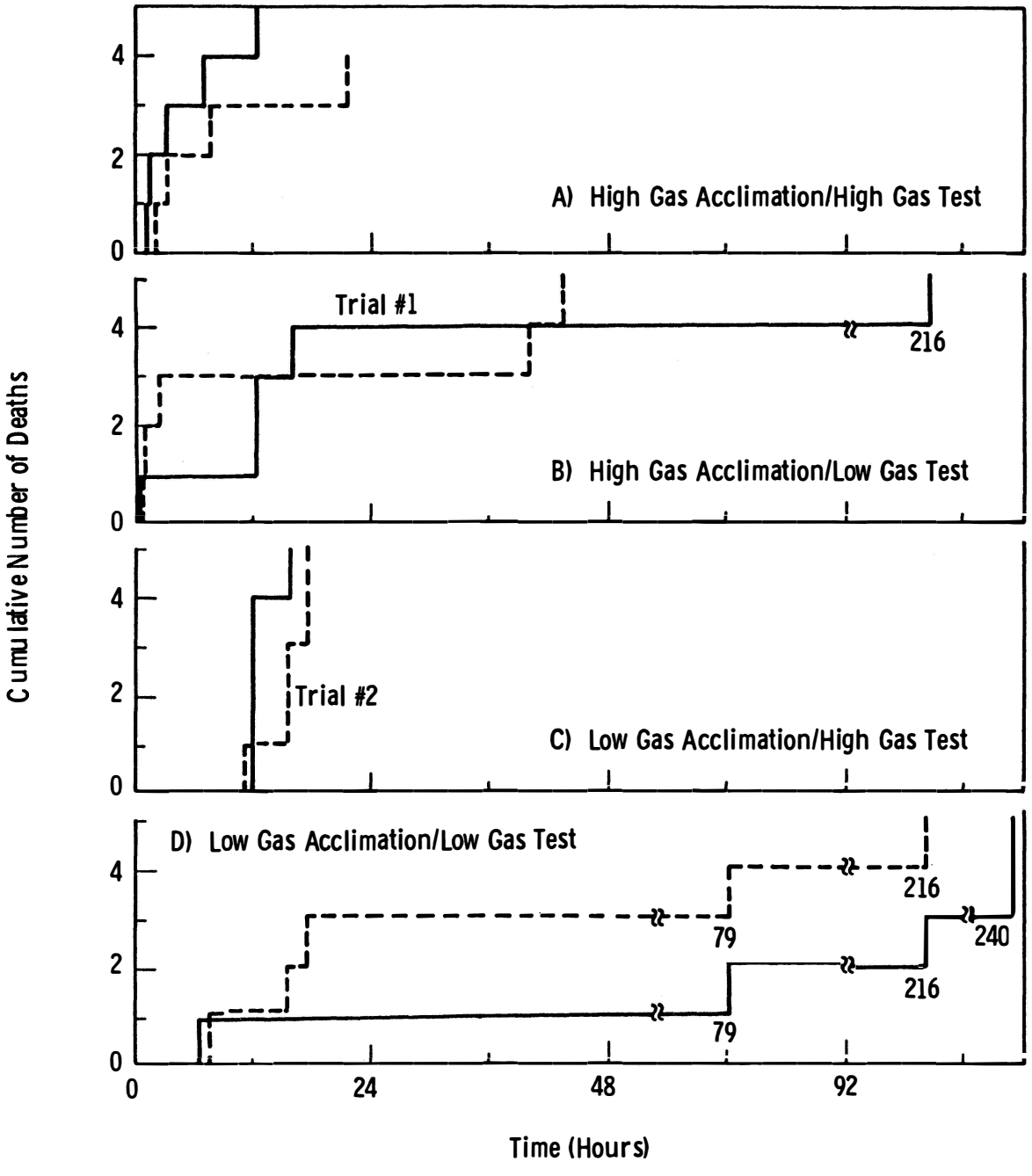

Fig. 8. Cumulative mortalities of test fish.

about 5 hours, while large ones there and elsewhere lost much tension. Large bubbles on the opercula remained for nearly 24 hours. By 24 hours following death, only a surface roughness requiring careful examination remained in areas once profuse with blisters. Sliming of dead fish made affected areas difficult to find unless the sites were known beforehand.

DISCUSSION

Previous studies of gas bubble disease do not suggest that nitrogen alone is responsible for gas blisters. Engelhorn (1943) analyzed the gas contents of bubbles in the fins of two species of freshwater fish finding that the initial gas concentrations reflected the composition of gas under artificial pressure. Several hours later, the percentage of nitrogen showed sharp increases due, Engelhorn suggests, to tissue respiration of the entrapped oxygen. Shirahata (1966) found the composition of abdominal bubbles of juvenile fish to be essentially that of air.

It seems most reasonable, therefore, that nitrogen saturation values determined for this test represent <u>air</u> saturation values. Some reduction of high oxygen tensions could, however, have been affected by fish respiration leaving inert nitrogen as the predominant effective gas.

Attempts to acclimate half of the test fish in high gas saturations produced debilitating chronic effects both through bubble symptoms and also perhaps through secondary infection. "Acclimation" was, therefore, never reached. This observation is contrary to findings of early European investigators (e.g. Engelhorn, 1943) who believed that sudden change in gas tensions were required for development of symptoms. Chronic effects of supersaturated gasses, especially oxygen, have received more consideration

in recent reports, and the effects are generally acknowledged. It is quite certain that the general poor condition of those experimental fish held for 6 to 9 days at high gas saturations (as indicated by accelerating mortalities and degenerating physical appearance) influenced the outcome of the tests reported here. The extent of this influence is difficult to assess, however. The fact that fish from the high gas acclimations died most quickly in all tests might be the result of their existing heavy stress. Some were able, however, to outlast low gas acclimated fish subjected to high test saturations. Subsequent experiments on this subject should consider a shorter acclimation time or a gas saturation level which is not itself so near a lethal level. Further work on the lethal action of gas saturation alone is suggested. It is further recommended that a single acclimation time be used, even when fish must be shipped over a period of several days. Our marked success at holding fish in water treated to remove supersaturated gasses suggests that all fish should be placed in this water until shipments are complete, followed by simultaneous transfer to acclimation tanks.

Rapid external drop of gas tension most closely approximating early experiments in the literature occurred in our transfer of high gas acclimated fish to the low gas test tank with a concurrent 5^{o} C temperature elevation. Rapid mortalities and evident distress by all fish shown in this treatment could have been due to sudden creation of internal gas embolisms, although no pathological examinations were made to substantiate this possibility. Since distress symptoms occur quite rapidly, it is tempting to speculate that they may be sufficient to cause avoidance reactions. Such avoidance might thereby block migration when an

area is encountered with lowered gas saturation values and higher temperatures. Such conditions were associated, perhaps coincidentally, with an apparent blockage of migration at the mouth of the Snake River in the summer of 1967 (Ebel and Johnson, personal communication).

The elevated temperature (22° C) chosen for interaction with gas saturation effects in these exploratory experiments was below the median one-week lethal level (about 25° C) for chinook salmon as determined by Brett (1952) for juveniles at acclimation temperatures comparable to those used here. There are no published data on the thermal sensitivity of adults or jacks of this species. The lethal level for adults may be less than that for juveniles and close to 22° C, however. This is suggested by the results of treatment (D) in which fish were subjected to temperature rise alone, without high gas saturations either during acclimation or during the test. In the second trial three of the five fish died within 24 hours; in the first trial, one fish had died in this time. Since the fish of the second run died faster throughout the test than those of the first, it is possible that there had been general deterioration of all fish with holding, or some detrimental effects of handling during transfers, despite their apparent good condition. Upstream migrating salmon generally survive 22° C water temperature in the lower Snake River, Washington in summer. It is also possible that more continuous monitoring of gas saturation values in test and acclimation tanks would have exposed some supersaturation where none has been thought to have existed. High cost of analysis, however was restrictive in this experiment.

The complete mortality within 24 hours of all fish in the high gas test treatment at 22° C, regardless of gas acclimation condition, is in sharp contrast to slower mortalities in low gas test treatments (excluding those rapid deaths noted above). It would appear that while a temperature of 22° C is questionably lethal acting alone, it is most certainly lethal when acting in combination with elevated gas saturations.

Previous acclimation to high gas saturations provides no resistance. In fact, the chronic gas bubble effects accumulated during acclimation seem to reduce survival time appreciably.

The significance of observations of the lability of gas bubble symptoms to field investigations of fish kills is readily, if disappointingly, evident. Fish having died more than 24 hours before collection will almost certainly have so few external gas bubble symptoms that these will be overlooked. Even after 6 hours, the profuse blisters on fins, which are the most evident external symptoms in many cases, will have nearly disappeared. No attempt was made at histological examination of internal organs.

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