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ABSTRACT

Tests were conducted using continuous exposure in shallow water at levels of total dissolved gas pressure ranging from 110-140% of barometric pressure (hyperbaric pressure = 103-410 g/cm^2). Both times to 20% and to median mortality were determined on several life stages of Pacific salmonids (Oncorhynchus and Salmo) and largemouth bass (Micropterus salmoides). Mean times to 20% mortality at 115% total gas saturation were 309, 154, and 125 hours for adults, smolts and parr. At 120% saturation mean times to 20% mortality were 48, 41, and 53 hours for adults, smolts, and parr. At 125% saturation, mean times to 20% mortality decreased to 18, 17, and 24 hours for adults, smolts, and parr. Factors which influence time to death included genera, life stage, acclimation temperature, activity level, sex, and body size. Mortality curves were typically skewed to the right. Gross pathology of gas bubble disease was described relative to these experiments. High gas levels that killed 50% of three species of salmon smolts had no apparent effect on the ability of survivors to tolerate an immediate transfer into seawater (30 ppt Cl). Long-term (3-month) continuous exposure of adult spring chinook salmon to 110% saturation had no readily apparent adverse impact on the fertilization and hatching of their eggs.

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ACKNOWLEDGEMENTS

We gratefully acknolwedge the assistance of the following agencies: Fish Commission of Oregon, Wildlife Commission of Oregon, National Marine Fisheries Service, U.S. Army Corps of Engineers, and the Columbia River Basin Interagency Fisheries Technical Advisory Committee.

SECTION I

INTRODUCTION

Scientists have studied supersaturation and its pathologic result, Gas Bubble Disease (GBD), for over 100 years with recurrent cycles of interest. Within the last decade interest has been renewed because man's activities have supersaturated large volumes of surface water, making them potentially lethal to fish (Ebel, 1969; Bouck, 1972). Causes of excess dissolved gas pressure (Table 1) include operations at hydroelectric or other dams, heating of water, and eutrophication (photosynthesis). Since most means of diminishing supersaturation could involve large financial costs, there must be a firm data base for establishing construction priorities or the necessary levels of water treatment and its achievable benefits.

An additional impetus for renewed research has been the development of improved techniques such as gas partition chromatography and the Weiss Saturometer which permit rapid analysis of total and individual dissolved gases. One might now resolve long standing questions regarding the biological impact of individual gases and easily monitor supersaturation in the aquatic environment.

The condition causing gas bubble disease has several names including supersaturation, air supersaturation, and nitrogen supersaturation. The term "air supersaturation" (Table 1) is used here because it correctly implies the typical problem, namely that the sum of the pressures of the dissolved components of air exceeds the combined compensatory pressures of the atmosphere, water, and tissues. Boyle's Law prescribes that gases will not cavitate regardless of how supersaturated a given gas may be, unless the total dissolved gas pressure (tension) exceeds the compensatory pressures.

The primary goal of this study was to obtain data on the acute lethality of supersaturated water to Pacific salmonids and a predator species (largemouth bass). These data were needed to evaluate what criterion of saturation (relative to barometric pressure 1/) would adequately protect fish and aquatic invertebrates. Shallow water and continuous exposure were selected for test conditions because they

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1/ Dissolved gas(es) levels conventionally are described as a percentage of some reference value, typically barometric pressure which varies continuously due to weather and between locations due to altitude. Continued use of this convention adds a potentially significant error to the data. This is because the relative value of 110% saturation can represent a (surface) uncompensated absolute force ranging from about 77.2 Kdynes at 7200 ft. above sea level to about 101.2 Kdgnes at sea level. However, abandoning this convention might seriously confuse engineers and biologists who are not associated with this research. The conversion of relative saturation levels to absolute physical units is presented in Table 4.

TABLE 1. EXAMPLES OF NATURAL AND MAN-MADE SOURCES OF SUPERSATURATED WATER

		Type No. I AIR ENTRAINMENT		Type No. II THERMAL		Type No. III ORGANIC
A. Natural	1.	Water falls with deep plunge basins.	1.	Cold water recharging an aquifer or lake, then geothermally warmed, then returned to surface.	1.	Eutrophic levels of photosynthesis in conjunction with high levels of temperature and solar radiation.
	2.	Turbulence in high velocity streams.	2.	Hot weather, often preceded by cold rain.		
B. Man-made	1.	Flood gates or other spillways at dams which entrain air bubbles and carry them to depths.	1.	Heating water to cool steam-electric stations or other industrial processes.	1.	Same as above except may occur in polluted water to a much greater degree.
	2.	Injection of air to prevent "water hammer" in turbines, sluiceways, or to reaerate reservoir water.	2.	Heating water in fish culture stations to achieve optimal growth.		
	3.	Venturi action at pipe joints, or pumps sucking air.				

represented the maximum environmental stress, eliminated significant hydrostatic compensation, and allowed a more direct comparison of tolerances between species. Collateral goals were to identify factors which might diminish tolerance to gas bubble disease, to determine residual effects on salt water adaptation, and to estimate if reproduction was readily and adversely affected by supersaturation.

The literature on Gas Bubble Disease has been reviewed recently by Harvey (1975), Wolke, Bouck and Stroud (1975), Bouck (1973), Weitkamp and Katz (1973), and Rucker (1972). These publications contain additional resource material and more detailed descriptions of Gas Bubble Disease.

SECTION II

CONCLUSIONS AND RECOMMENDATIONS

1. Some mortality occurred among sensitive fish at dissolved gas levels of 110-115% of barometric pressure when they were restricted to shallow water. These and higher gas levels may be safe for wild fish if they sound to compensatory depths.

2. The mean hours to 20% mortality and two-tailed 90% confidence limits are:

Salmonids*	Supersaturation Level 115%	120%	125%
Adults	309 (± 114)	48 (± 8.7)	18.3 (± 3.2)
Smolts	154**	41 (± 10.1)	17.2 (± 5.2)
Juveniles	125 (± 122)	53.5 (± 23.8)	23.6 (± 4.4)

*(10 C, shallow water)
**(= single sample only)

3. Response (time to death) curves are skewed to the right and contain at least three phases which include: (1) a sublethal period; (2) a period of log-linear mortality; and (3) a period of protracted survival. These skewed curves indicate that: (1) as much as 75% of a laboratory population of fish can be killed in about half the time required to kill the total population; (2) probably few factors influence the mortality; and (3) the median is probably the best measure of central tendency in these data.

4. Times to death varied among the tests, genera and species in some cases. Variability was greater in long-term exposure tests than in short, acute exposures.

5. Several additional factors were significantly correlated with time to death. These included life stage, physiological condition, body size, activity, behavior, and water temperature.

6. Largemouth bass survived prolonged exposure at 120% of barometric pressure (203 g/cm^2 hyperbaric) which killed salmon and trout. This level caused external signs of gas bubble disease on bass but did not prevent them from preying on young salmonids. Thus supersaturation may exert ecological succession pressure against salmonids and favor bass.

7. The external lesions of gas bubble disease such as dermal emphysema or exophthalmus varied between species and became most severe when conditions were marginally lethal; such conditions allowed the longest survival, hence the longest period for the pathologic process to develop. In acutely lethal cases with short exposure time, early mortality frequently showed no external signs of gas bubble disease. Therefore, the presence of air emboli in the blood along with identification of supersaturated water must be used to confirm acutely lethal gas bubble disease.

8. Supersaturation levels which killed 50% of the smolts in a species did not prevent the survivors from tolerating a direct transfer to 30 $^{o}/oo$ seawater where they survived for five days.

9. Appearance, fertility and hatching of eggs were not adversely affected in spring chinook which had been held at 110% for three months (10 C, 65 cm of water) and artifically spawned.

10. Actual conditions encountered by fish in supersaturated water should be clearly defined, such as intermittent exposure and water depth.

11. A zero supersaturation limit is recommended for salmonid fish hatcheries; any supersaturation typically indicates a more basic problem that is capable of reaching harmful levels especially when the water is heated or pumped.

SECTION III

METHODS AND MATERIALS

SUPERSATURATION EXPOSURE FACILITIES

Each exposure chamber operated independently and consisted of a large fiberglass tank about 3.75 m in diameter by 1 meter deep. A frame supported rubber-coated nylon sides (1.2 m high) and 2.5 cm mesh netting over the top. These tanks were located outdoors, but insulated with 5 cm of urethane foam and located under a roof to shield them from direct sunlight (Figures 1 and 2).

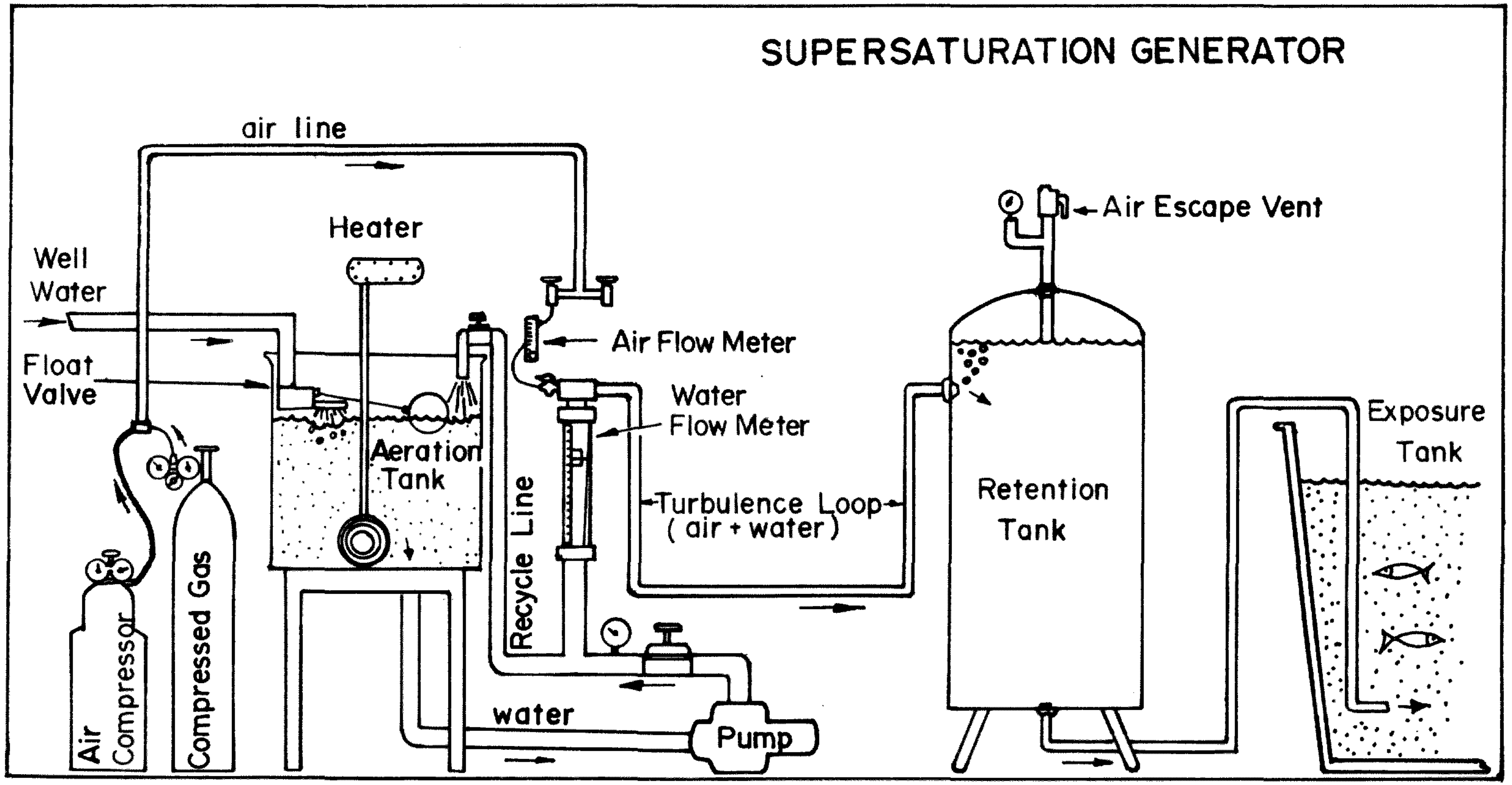
Well water was aerated to near saturation before being pumped at 1900 kilo dynes/cm^2 through each exposure system. A small amount of this water was recycled to the aeration tank to promote aeration. Compressed air was injected at a constant rate into the pressurized water immediately prior to passing thru pipe ells (Figure 1) where turbulence promoted the solution of the gases. The water passed into a retention tank and the undissolved air was removed by drawing air off the top; water was drawn off the bottom and delivered to the test tank. The level of total dissolved gas pressure (supersaturation) was proportional to the volume of air being injected into the system. As tested, this system could deliver 75 l/min of water with a total dissolved gas pressure easily as high as 150 percent of barometric pressure. Water was introduced into the exposure chamber via a subsurface port to reduce gas losses to the air. Valves were omitted wherever possible to avoid sharp drops in pressure which remove gases from solution.

Additional exposure chambers were located within a circular cage made of wood, coated with epoxy paint, and covered with nylon bobinet (Figure 2 G). Each cage, about 1.3 m in diameter and 1 m high, was equipped with hinged doors and sub-divided into quadrants making four additional exposure chambers. The cage assembly sat over and was held in place by the tank stand-pipe. Water currents within the cage promoted good mixing of water, but velocities were slower in the cage than in the open tank.

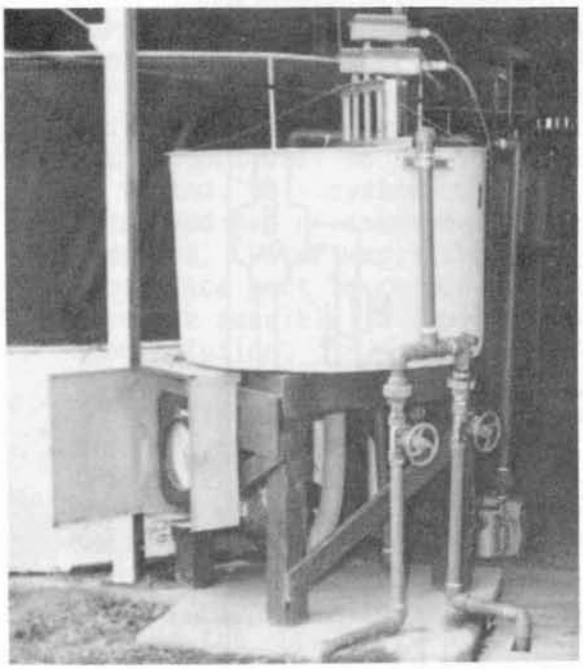
WATER QUALITY

Water for these experiments was drawn from a well located adjacent to the Willamette River. Water from this source has been used for fish culture for five years, and its general characteristics are listed in Table 2. Hardness and alkalinity were typically low (20 ppm as $CaCO_3$) and ranged between pH 7.0-7.4 after aeration. Water temperature was kept at 10 C except where it was altered as an experimental variable.

FIGURE 1. DIAGRAM OF FACILITIES FOR EXPOSING FISH TO SUPERSATURATED WATER.



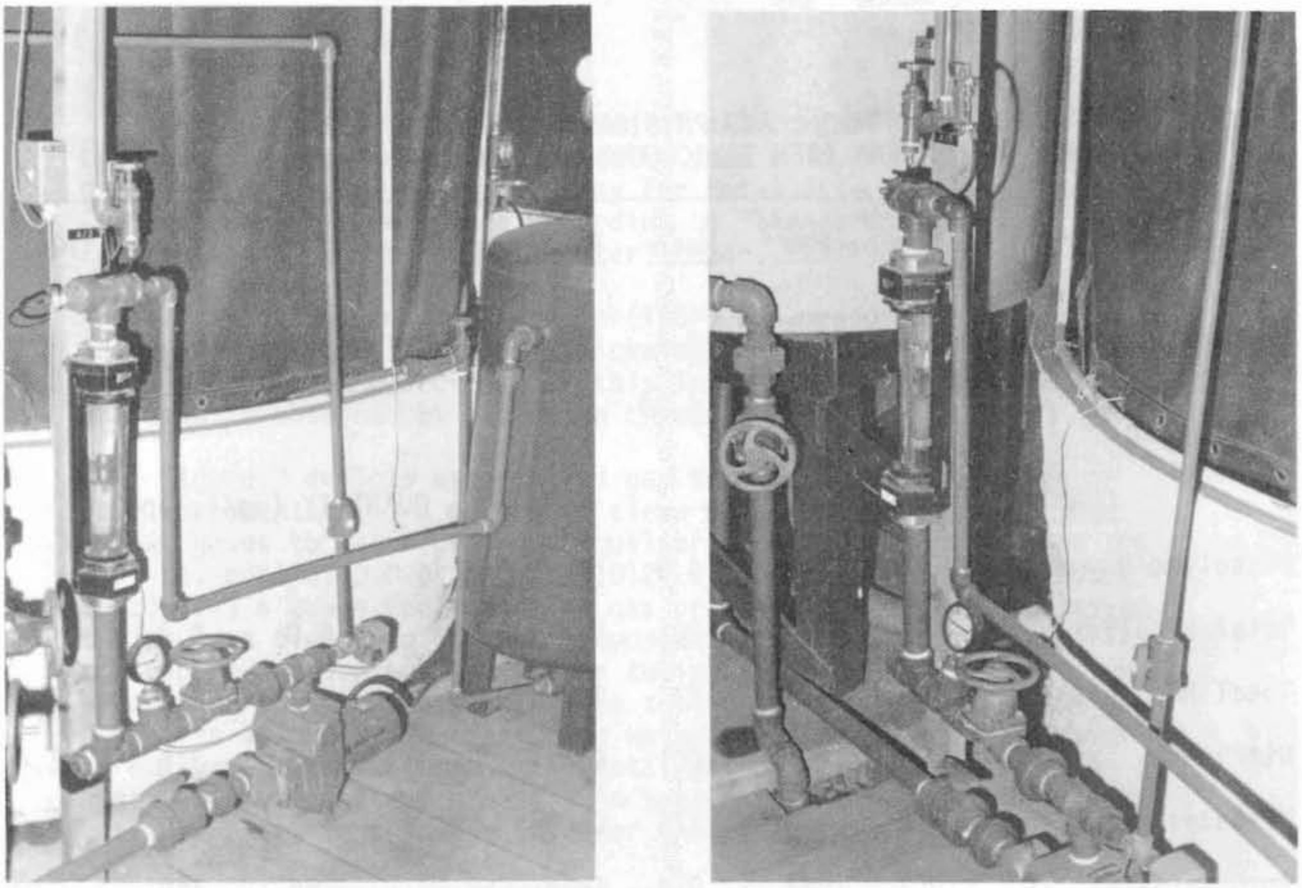
A--Test tanks with roof to shield out direct sunlight.



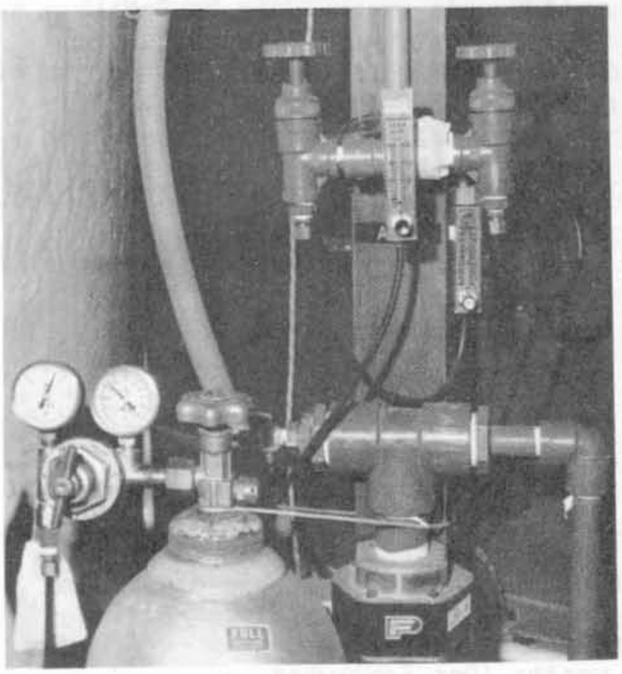
B--Aeration tank with incoming water supply and continuous temperature control system.

C--Test tank with saturometer in preparation for gas analysis.

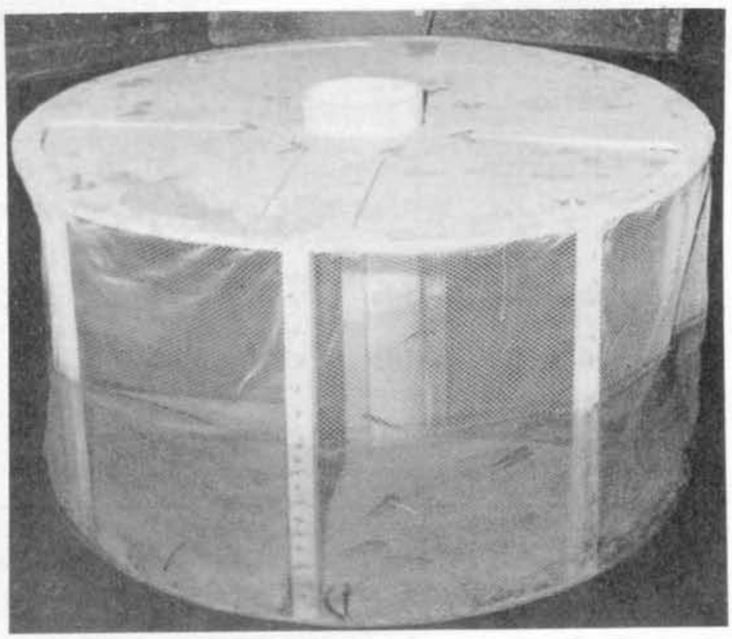
Figure 2. Supersaturation exposure facilities



D (left), E (right)--Supersaturation generator system (see Figure 1).



F--Air and/or compressed gas delivery system of supersaturation generator.



G--Four-chambered nylon bobinet cages within the test (exposure) tanks. Young sockeye salmon can be seen in the chambers.

Figure 2 (continued). Supersaturation exposure facilities

TABLE 2. A SUMMARY OF CHEMICAL AND PHYSICAL PARAMETERS IN THE WATER SUPPLY OF WESTERN FISH TOXICOLOGY STATION 1972-1973[1].

Parameter[2]	Min	Max	Mean	Parameter[2]	Min	Max	Mean
Temperature (°C)	7.0	18.2	13.1				
pH	6.70	7.00	6.80				
QUANTITY (mg/l, ppm)				QUANTITY (μg/l, ppb)			
Dissolved Oxygen	2.8	9.0	4.9	Heavy Metals			
Total Alkalinity	18	26	22	Cadmium	0.0	0.3	0.1
Total Hardness	21	25	23	Chromium	1.2	5.0	1.3
Nitrites	<.001	<.001	<.001	Cobalt	0.5	3.0	1.4
Nitrates	.138	.330	.176	Copper	1.0	9.0	3.5
Ammonia	<.001	.048	.014	Iron	55	360	110
Calcium	5.8	13.7	7.3	Lead	3.0	5.0	4.7
Magnesium	1.4	2.4	1.7	Magnesium	1.0	22.0	2.9
Sodium	4.1	5.4	4.6	Nickel	1.0	4.0	1.9
Potassium	0.48	0.70	0.57	Zinc	1.0	24.0	6.4
Sulfate	2.0	4.0	2.9	Mercury	<0.5	2.0	0.7
Dissolved Solids	34	68	52				
Suspended Solids	<1.0	4.0	1.7				
Chloride	3.0	9.0	6.1				

1/ Unpublished Data. Donald F. Samuelson

2/ Number of samples for all parameters ranged between 23-26. Maximum values occurred during winter months (Dec-Feb) when saturated ground water dominated the river water supplying our well.

Water quality in the experimental and control tanks was monitored continuously for temperature; usually once a day for pH, alkalinity and oxygen; and several times each day for total dissolved gas pressure. Chemical analyses were done according to "Standard Methods for the Examination of Water and Wastewater" (Anon., 1971).

Total dissolved gas pressure (TDGP) was measured with a dissolved gas tensionmeter1/ (Table 3). Fickeisen, Schneider and Montgomery (1974) reported the accuracy of this instrument to be comparable to the results obtained by partition chromatography.

Figure 3 depicts a dissolved gas tensionometer consisting of four components: (1) a molecular sieve which excludes liquid water but allows gases to reach pressure equilibrium with their dissolved phases (400 ft. coil of 0.025" O.D. X 0.012" I.D. dimethyl silicone rubber tube); (2) a gauge for measuring gas pressure or tension; (3) nylon tubing (gas tight) to connect components (1) and (2); and (4) a framework to support and protect the rubber tubing. The sensor is placed under water, and the gas pressure in the tubing equilibrates with the gas pressures in the water, including water vapor pressure. The gauge directly measures the resulting total gas pressure, indicating a hyperbaric or positive pressure in supersaturated water and a hypobaric or negative pressure in water that is not fully saturated.

The measurement of dissolved gas pressure is based on the principle that the membrane is selectively permeable to gases but not to liquids. A dissolved gas crosses the membrane and in due time reaches a pressure equilibrium with its dissolved gas phase. The gauge sums the pressures of the individual gases including that of water vapor, but always relative to ambient barometric pressure. Therefore, this instrument measures the total pressure difference (ΔP) between barometric pressure and the sum of dissolved gas pressures including water vapor pressure.

The relative total dissolved saturation is calculated by the formula:

$$\frac{BP + \Delta P - VP}{BP} \times 100, \text{ where}$$

BP = barometric pressure in mmHg;
ΔP = differential gas pressure in mmHg (from saturometer);
VP = vapor pressure of water in mmHg.

Thus "percent saturation" is a term which relates to the local barometric pressure, which in turn changes with altitude and weather conditions. In our case, barometric pressure averaged about 755 mmHg

1/ Developed by Dr. Ray Weiss, Scripts Institute of Oceanography, LaJolla, California, and initially called a "saturometer".

TABLE 3. NOMINAL AND MEASURED TEST LEVELS OF TOTAL GAS SUPERSATURATION.

Test No.	Test Chamber	Nominal Total Gas Saturation (%)	Measured Total Gas Saturation (%) N	Mean	Range
1	1	125	16	125.2	123.6 - 127.6
2	1	120	59	120.1	116.6 - 123.7
3	1	125	31	125.5	122.7 - 127.8
4	1	120	44	120.2	115.2 - 123.5
5	1	115	42	115.6	114.0 - 125.0
6	1	125	43	125.0	123.5 - 127.7
	2	120	53	120.1	118.2 - 122.2
7	1	120	37	120.3	118.3 - 122.6
	2	115	39	114.9	113.0 - 116.3
8	1	125	45	124.7	123.2 - 126.3
9	1	125	43	124.8	122.6 - 126.3
	2	120	42	120.1	118.7 - 121.0
10	1	140	20	139.3	137.2 - 141.0
	2	130	30	129.7	128.2 - 130.8
11	1	120	24	120.6	117.7 - 122.0
	3	115	34	114.7	112.6 - 116.5
12	2	140	8	140.6	139.9 - 141.0
13	1	140	16	140.7	138.4 - 142.4
	2	130	22	130.1	127.5 - 133.4
14	1	135	7	136.0	133.3 - 138.5
15	1	140	10	141.2	138.9 - 143.2
16	1	120	10	120.0	117.4 - 121.1
	2	115	21	115.2	113.5 - 116.9
	3	110	19	109.8	109.1 - 110.6
17	1	120	7	119.8	119.1 - 120.5

TABLE 3. NOMINAL AND MEASURED TEST LEVELS OF TOTAL GAS SUPERSATURATION (CONTINUED).

Test No.	Test Chamber	Nominal Total Gas Saturation (%)	Measured Total Gas Saturation (%)		
			N	Mean	Range
18	1	125	7	125.5	123.9 - 127.2
19	1	130	17	130.4	126.4 - 133.0
	2	125	17	125.0	123.3 - 127.2
	3	120	43	119.8	115.1 - 123.4
20	1	120	35	120.2	117.1 - 121.9
	2	115	59	115.0	111.9 - 117.3
	3	110	157	109.8	107.0 - 116.5

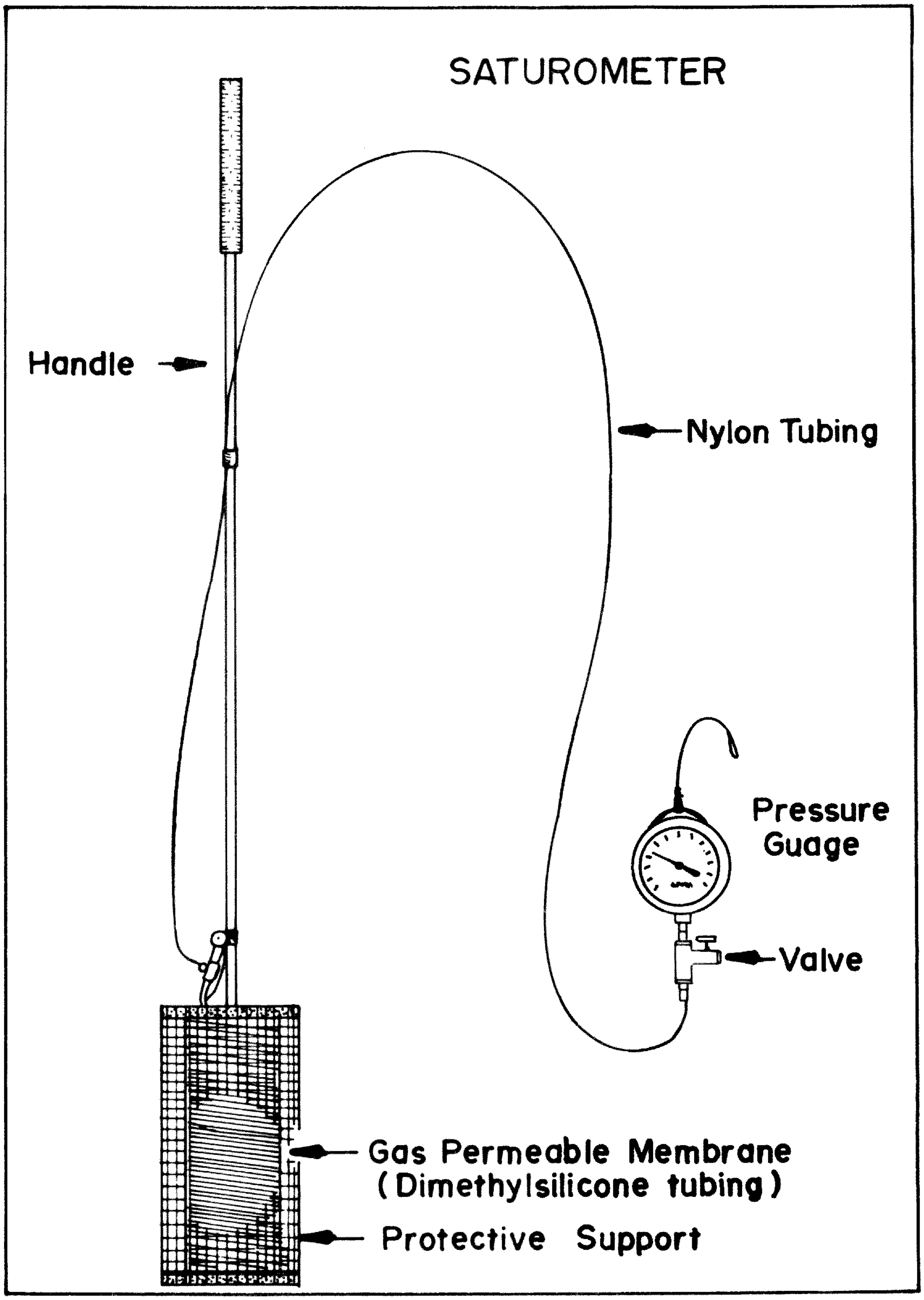


Figure 3. Diagram of modified Weiss Saturometer.

and varied typically by less than 20 mmHg (2.6% of average). However a given "total" saturation value does not imply that each component gas was saturated to that level. For example, a total saturation of 110% can consist entirely of high N_2 levels and low O_2 levels or vice versa.

Since the causative agent of gas bubble disease is related (in part) to the dissolved gas pressure above barometric levels (Bouck, 1973; D'Aoust and Smith, 1974), this is called the ΔP or hyperbaric pressure, and it can be measured both accurately and directly with the saturometer. The ΔP is the uncompensated hyperbaric pressure that provides the driving force to form emboli and emphysema by overcoming the compression forces in blood and other tissues. Hyperbaric pressure may prove to be a more universal and determinative parameter than "percent saturation" because it is independent of elevation, barometric pressure, salinity, or other influences. Table 4 lists equivalent values for percent saturation, g/cm^2, mmHg, and p.s.i.

BIOLOGICAL METHODS

Fish for these experiments were obtained from several sources listed in Table 5. All juvenile fish were hatchery reared, mainly at this laboratory, but some adult fish were returnees to hatcheries. Largemouth bass, sockeye, and possibly spring chinook salmon were wild fish. Fish usually were placed into the exposure tank and allowed to adjust to their surroundings for at least 48 hours prior to the onset of supersaturation. Food was routinely withheld during each experiment, but some exceptions are so noted. Adult fish were tested in the open tanks, but smaller fish were usually tested in the bobinet cages (Figure 2).

Common and scientific names of fishes conform to that of Bailey, et al., (1970).

Adult salmon were spawned artificially by the "dry" method after they ripened sexually (Leitriz, 1959). Eggs were incubated in tray type incubators. Dead and infertile eggs were removed after pigment was evident in the embryonic eye and again after hatching was complete. Temperatures varied seasonally during incubation from 10 C down to about 6 C.

DESIGN OF EXPERIMENTS

The three objectives were to: (1) determine and compare the exposure times (in hours) which killed 50% of the specimens at given test conditions; (2) determine the effects of supersaturation on saltwater adaptation; and (3) determine the effect of long-term exposure to low

TABLE 4. CONVERSION TABLE AND EQUIVALENT UNITS FOR EXPRESSING SATURATION DATA

Percent saturation at 755 mmHg	Hyperbaric Pressure			
	g/cm^2 1/	Torr or mmHg	lb/in^2 (psi)	Bars 2/
100	0	0	0.00	0
105	51.4	37.8	0.75	0.05
110	102.6	75.5	1.47	0.10
115	153.9	113.2	2.21	0.15
120	205.3	157.0	2.94	0.20
125	256.7	188.8	3.68	0.25
130	307.9	226.5	4.41	0.30
135	359.2	264.2	5.15	0.36
140	410.6	302.0	5.88	0.40
145	461.9	350.0	6.62	0.46
150	513.6	377.5	7.35	0.50

1/ To convert g/cm^2 to dynes, multiply by 980 cm/sec^2 (which is the approximate acceleration of gravity)

2/ Bars are one of two pressure units recommended for use by the Society for Experimental Biology and Medicine (1972):

1 bar = 10^5 Pascals = 14.5 lbs/in^2 = 0.987 atmospheres

TABLE 5. FISH SPECIES TESTED

Species	Test No.	Life Stage	Source
Coho Salmon	6,7	parr*	Oregon Fish Commission Hatchery
(*Oncorhynchus kisutch*)	6,7	post smolts*	Fall Creek, Lincoln Co., Oregon
	6,7	jacks	
	6,7	adults	Bonneville Hatchery - Ore. Fish Comm.
Sockeye Salmon	4,5,7	parr*	Columbia River, Bonneville Ladder
(*Oncorhynchus nerka*)	16,17,18	smolts*	
	1,2,3,4,5	adults	
Chinook Salmon	20	parr*	Oregon Fish Commission, Willamette
spring migrating variety	16	smolts*	Hatchery, Oakridge, Oregon
(*Oncorhynchus tshawytscha*)			
	19,20	adults	Columbia River Bonneville Ladder
Steelhead Trout	8	parr	Washougal River Hatchery,
(*Salmo gairdneri*)	16	smolts	Washington Game Commission
summer race	1,2,5	adults	Columbia River, Bonneville Ladder
Winter race	7,8	parr*	Oregon Wildlife Commission Hatchery
	16	smolts*	N. Fk. Alsea R., Benton Co., Oregon
	11	adults	
Rainbow Trout	9	parr*	Oregon Game Commission, Roaring
(*Salmo gairdneri*)	3,4,5,9	yearlings*	River Hatchery, Linn Co., Oregon
Suckers	20	adults	Columbia River at Bonneville
(*Catostomus sp.*)			
Largemouth Bass	9,10,12,13,14,15	juveniles	Ponds, Harrisburg, Lane Co., Oregon
(*Micropterus salmoides*)	9,10,12,13,14,15	adults	

* Reared from eggs at WFTS.

level supersaturation on maturation and fertilization of sex products and subsequent hatching success. Collateral objectives included the identification of factors influencing mortality and observations of the pathobiology of gas bubble disease.

Fish were assigned randomly to tanks, and a fish was judged to be dead when it no longer moved or ventilated. Eight levels of saturation were selected for testing: 100 (control), 110, 115, 120, 125, 130, 135, and 140% of barometric pressure (hence total gas saturation). Circumstances never permitted the concurrent testing at every level, but a control group and at least one previously tested level were repeated in a series. Replicates or re-runs were conducted whenever the circumstances permitted, and several life stages or species were tested concurrently in most cases. The fish were observed frequently, and times to mortality were plotted on log probit paper for visual estimation of the time to median mortality (ET_{50}). Raw data were plotted and used to estimate the relationship between various levels of supersaturation and respective times to 20% and median mortality. For the latter purpose all salmonid data were pooled for a given life stage.

To study tolerance to the combined stresses of supersaturation followed by saltwater stress, smolts of three species were exposed to three levels of supersaturation for four days at 10 C or until 50% mortality occurred. Immediately thereafter, the survivors were placed in 20 liter jars containing seawater (30 $^{o}/oo$) and equipped with an air supply and refrigeration to maintain 10 C ± 1°. Survival was noted at the end of a five-day period.

The effects of sublethal supersaturation upon sexual maturation, fertility, and hatching were studied using adult spring chinook trapped in the Columbia River. These were held at 110% from April 23, 1973 until August 3 (103 days), then maintained at normal gas levels for about three weeks before the fish were artificially spawned.

The general effects of temperature on mortality were studied at 10, 15, and 18 C. The fish were adjusted at a rate ≤ 3 C/day until they reached the test temperature. Fish were maintained at the test temperature for at least two days prior to initiating supersaturation.

STATISTICAL PROCEDURES

Fish were assigned to treatments using prepared random number cards. All statistical procedures conform to those of Snedecor and Cochran (1973). Statistical significance was assigned whenever the probability of obtaining a given result by chance alone was <.10.

SECTION IV

RESULTS

GENERAL TOLERANCES OF SALMONIDS AND NON-SALMONIDS

Preliminary concurrent exposure of several representative species, conducted at 130-150% saturation (10 C), established that salmonid species (Salmo and Oncorhynchus) were highly susceptible to gas bubble disease. Shiners (Notropis sp.) and crappies (Pomoxis sp.) also died rapidly under those conditions. Bluegill (Lepomis macrochirus), squawfish (Ptychocheilus oregonesis), and warmouth (Lepomis gulosus) were intermediate in tolerance to bullhead (Ictalurus sp.), largemouth bass (Micropterus salmoides), and carp (Cyprinus carpio) which were extremely hardy under those test conditions. These results reveal differences in tolerance between genera and demonstrate that salmonids were both highly sensitive and easy to use in these tests.

SAMPLE RESPONSES TO SUPERSATURATION

A general estimation of population response (mortality) to supersaturation is essential to understanding the overall problem. Specific information on this subject has not been found in the literature: hence, we analyzed the data from twenty bioassays (each used either 10 or 20 fish per bioassay) and estimated the response curve. Individual times to death from experiments with 100% mortality constituted the raw data. Various measures of central tendency or dispersion were estimated for population parameters listed in Table 6.

As a preliminary step, pooled times to death data for all life stages were tested by analysis of variance, and this revealed that the samples were significantly heterogeneous at the 10% level for a given level of saturation. Subsequently the data were pooled only within a given life stage because gross differences in tolerance were apparent between life stages.

Curves plotted for cumulative mortality and rate of death (Figures 4 and 5) show three phases of population response to supersaturation. The initial phase consists of a sublethal morbid period during which emboli form and related dysfunctions approach critical limits for fish life. The second phase is a period of extensive mortality which appears linear with the $\log_{10}$ of exposure time. The third phase did not occur in all samples nor at all saturation levels, although its intermittent occurrence indicates likelihood if larger samples or more replicates were tested for a longer time. During the third phase the more tolerant fish died at a greatly reduced rate, typically over an extended period of time. This protracted survival period gave many fish samples a definite and significant skewness that rejects the hypothesis of random normal distribution. Possibly a fourth phase may exist consisting of post-exposure mortality or recovery from all effects that carried over after returning to normal saturation levels.

TABLE 6. SUMMARY STATISTICS OF TIME TO DEATH AT THREE LEVELS OF SUPERSATURATION BY TWENTY POPULATION SAMPLES OF RAINBOW TROUT, PACIFIC SALMON, AND LARGEMOUTH BASS.

	Number in Sample	Range (hours)	Average (hours)	90% Belt for μ	Median (hours)	Standard Deviation	Coefficient of Variation	Coefficient of Skewness
10C, 130% Supersaturation								
1. Spring chinook salmon (*Oncorhynchus tshawytscha*) ($\bar{X}$FL = 81.0cm; $\bar{X}$Wt = 7.738kg) adult female, migrating								
a. Time to death	11	7-12	8.909	±0.929	8.5	1.700	19.0	+0.160
b. Log_{10}	-	0.845-1.0792	0.943	±0.044	0.954	0.082	8.7	+0.419
2. Spring chinook salmon (*Oncorhynchus tshawytscha*) ($\bar{X}$FL = 60cm; $\bar{X}$Wt = 5.618g) adult male, migrating								
a. Time to death	9	7-11	9.440	±0.701	10	1.130	12.0	1.486
b. Log_{10}	-	0.845-1.041	0.972	±0.034	1.00	0.056	5.7	1.497
10C, 125% Supersaturation; 10C								
3. Rainbow trout (*Salmo gairdneri*) ($\bar{X}$FL = 15.2cm; $\bar{X}$Wt = 42.2g) parr								
a. Time to death	20	20-48	30.150	±3.276	27	8.474	28.1	1.115
b. Log_{10}	-	1.301-1.681	1.4645	±0.044	1.431	0.114	7.8	0.874
4. Rainbow trout (*Salmo gairdneri*) ($\bar{X}$FL = 28.18cm; $\bar{X}$Wt - 259.39) yearling								
a. Time to death	10	17-45	33.70	±5.556	31	9.696	28.8	0.835
b. Log_{10}	-	1.230-1.653	1.1510	±0.0792	1.4914	0.1385	12.0	7.3732
5. Winter steelhead trout (*Salmo gairdneri*) ($\bar{X}$FL = 7.8cm; $\bar{X}$Wt = 5.6g) parr								
a. Time to death	20	10-224	66.55	±24.34	35	62.966	94.6	1.360
b. Log_{10}	-	1.000-2.3502	1.6519	±0.966	1.5524	0.150	24.4	0.559
6. Sockeye salmon (*Oncorhynchus nerka*) ($\bar{X}$FL = 10.2cm; $\bar{X}$Wt = 12.0g) parr								
a. Time to death	20	13-189	62.150	±18.566	40.0	48.022	77.3	1.509
b. Log_{10}	-	1.0128-2.2765	1.6551	±0.1420	1.6021	0.367	22.2	0.615
7. Spring chinook salmon (*Oncorhynchus tshawytscha*) ($\bar{X}$FL = 79.6cm; $\bar{X}$Wt = 5.816kg) adult females								
a. Time to death	16	12-22	17.875	±1.357	18	3.095	17.3	0.605
b. Log_{10}	-	1.0792-1.3424	1.2456	±0.0346	1.2553	0.0798	6.4	0.810
8. Coho salmon (*Oncorhynchus kisutch*) ($\bar{X}$FL = 20.4cm; $\bar{X}$Wt = 87.5g) post smolts								
a. Time to death	20	6-21	12.500	±1.559	12.0	4.032	32.2	0.372
b. Log_{10}	-	0.7782-1.3222	1.0578	±0.0556	1.0792	0.1468	13.8	0.437

TABLE 6. SUMMARY STATISTICS OF TIME TO DEATH AT THREE LEVELS OF SUPERSATURATION BY TWENTY POPULATION SAMPLES OF RAINBOW TROUT, PACIFIC SALMON, AND LARGEMOUTH BASS (CONTINUED).

	Number in Sample	Range (hours)	Average (hours)	90% Belt for μ	Median (hours)	Standard Deviation	Coefficient of Variation	Coefficient of Skewness
9. Coho salmon (*Oncorhynchus kisutch*) ($\bar{X}$FL = 40.3cm; $\bar{X}$Wt = 795g) jacks (precocious)								
a. Time to death	20	12-21	17.200	±1.276	19	3.3015	19.2	1.635
b. Log_{10}	-	1.0792-1.3222	1.2275	±0.0054	1.2788	0.0865	7.0	1.780
10. Coho salmon (*Oncorhynchus kisutch*) ($\bar{X}$FL = 71.1cm; $\bar{X}$Wt = 4.173kg) adult female, gravid								
a. Time to death	10	15-28	20.900	±1.902	21	3.2812	15.7	0.091
b. Log_{10}	-	1.1761-1.4472	1.3153	±0.0392	1.3222	0.0683	5.2	0.301
11. Coho salmon (*Oncorhynchus kisutch*) ($\bar{X}$FL = 66.8cm; $\bar{X}$Wt = 3.678kg) adult male, ripe								
a. Time to death	10	12-42	23.2	±5.161	20	8.904	38.38	1.078
b. Log_{10}	-	1.0792-1.6232	1.3403	±0.1867	1.3010	0.1520	11.34	0.015
10C, 120% Supersaturation								
12. Rainbow trout (*Salmo gairdneri*) ($\bar{X}$FL = 15.3cm; $\bar{X}$Wt = 43.9kg) parr								
a. Time to death	20	32-193	70.650	±16.403	51	42.475	60.1	1.388
b. Log_{10}	-	1.5051-2.2856	1.7950	±0.0802	1.7076	0.2078	11.6	1.262
13. Winter steelhead trout (*Salmo gairdneri*) ($\bar{X}$FL = 69.2cm; $\bar{X}$Wt = 3.563kg) adult females, gravid								
a. Time to death	10	59-164	104.50	±19.316	92	33.323	31.9	1.107
b. Log_{10}	-	1.7709-2.2148	1.9987	±0.0797	1.9638	0.1377	6.8	0.1377
14. Winter steelhead trout (*Salmo gairdneri*) ($\bar{X}$FL = 70.2cm; $\bar{X}$Wt = 3.336kg) adult males, ripe								
a. Time to death	10	57-144	90.30	±16.599	79	28.6397	31.72	1.1837
b. Log_{10}	-	1.7559-2.1584	1.9370	±0.0766	1.8973	0.1324	6.83	0.8998
15. Coho salmon (Oncorhynchus kisutch) ($\bar{X}$FL = 73.0cm; $\bar{X}$Wt = 4.372kg) adult female, migrating, gravid								
a. Time to death	10	31-64	45.700	±5.178	45	8.9324	19.5	0.235
b. Log_{10}	-	1.4914-1.8062	1.6525	±0.0488	1.6532	0.0843	5.1	0.025
16. Coho salmon (*Oncorhynchus kisutch*) ($\bar{X}$FL = 70.4cm; $\bar{X}$Wt = 4.080kg) adult female, gravid								
a. Time to death	10	42-110	58.80	±11.348	51	19.577	33.2	1.1950
b. Log_{10}	-	1.6232-2.0414	1.7526	±0.0693	1.7076	0.1197	6.8	1.1288

TABLE 6. SUMMARY STATISTICS OF TIME TO DEATH AT THREE LEVELS OF SUPERSATURATION BY TWENTY POPULATION SAMPLES OF RAINBOW TROUT, PACIFIC SALMON, AND LARGEMOUTH BASS (CONTINUED).

	Number in Sample	Range (hours)	Average (hours)	90% Belt for μ	Median (hours)	Standard Deviation	Coefficient of Variation	Coefficient of Skewness
17. Spring chinook salmon (*Oncorhynchus tshawytscha*) ($\bar{X}$FL = 66.7cm; $\bar{X}$Wt = 5.208kg) adult female, migrating								
a. Time to death	9	22-99	54.44	±16.735	51	30.623	56.2	0.337
b. Log_{10}	-	1.3424-1.9956	1.6676	±0.1450	1.7076	0.2653	15.9	0.452
20C, 130% Supersaturation								
18. Largemouth bass (*Micropterus salmoides*) ($\bar{X}$FL = 22.4cm; $\bar{X}$Wt - 163.4g)								
a. Time to death	20	25-69	45.45	±4.782	45	12.369	27.2	0.109
b. Log_{10}	-	1.3979-1.8388	1.6424	±0.0454	1.6532	0.1175	7.2	0.274
20C 140% Supersaturation								
19. Largemouth bass, adults ($\bar{X}$FL = 23.1; $\bar{X}$Wt = 187.5g)								
a. Time to death	20	10-22	16.550	±1.3924	16	3.349	20.2	0.493
b. Log_{10}	-	1.000-1.3424	1.2098	±0.0381	1.2041	0.0924	7.6	0.183
20. Largemouth bass, adults ($\bar{X}$FL = 24.0cm; $\bar{X}$Wt = 209.6g)								
a. Time to death	20	11-19	12.45	±0.8551	11	2.2117	17.8	1.966
b. Log_{10}	-	1.0414-1.2788	1.0895	±0.0696	1.0414	0.0696	6.4	2.074

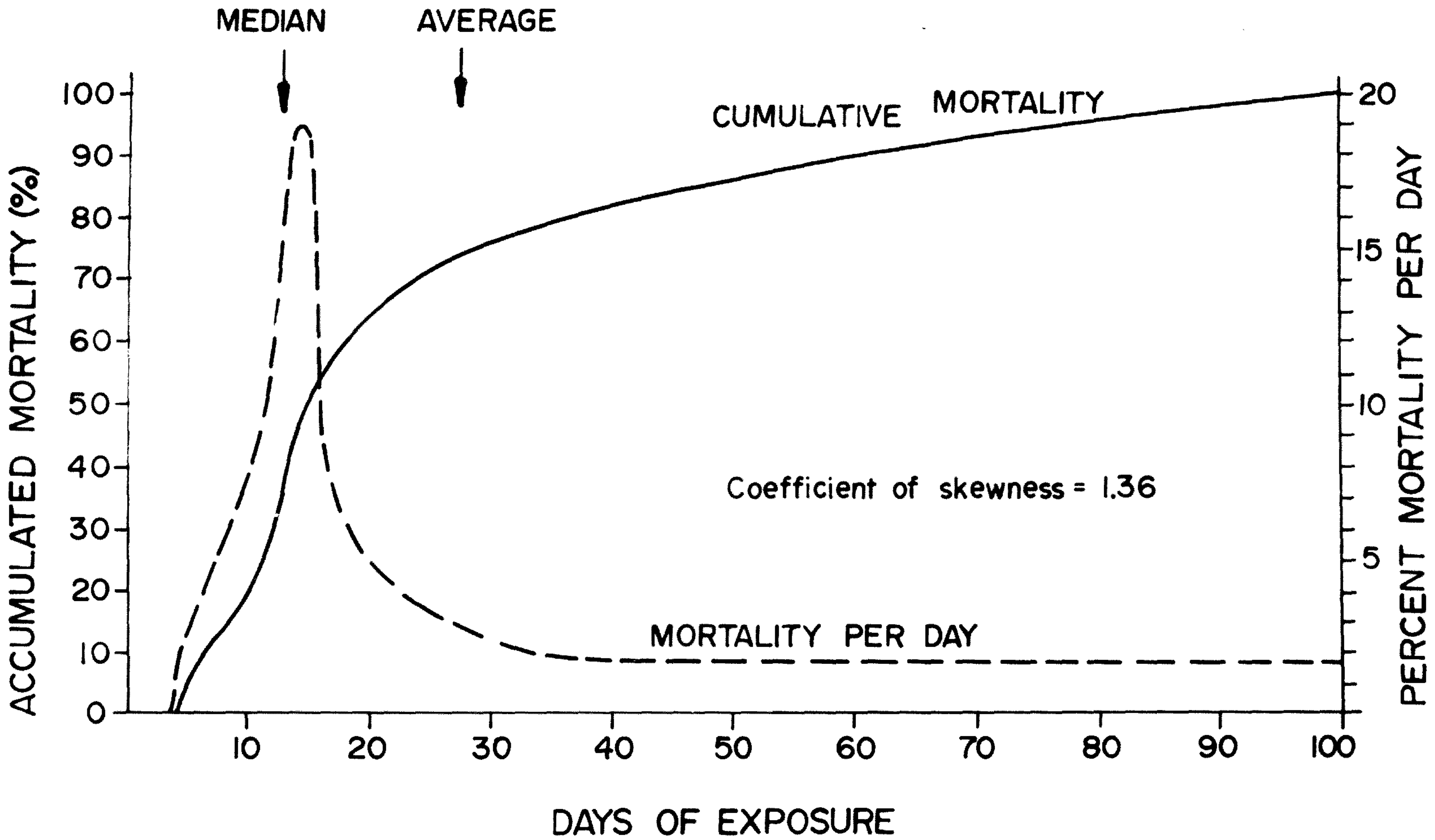


Figure 4. Time to death of winter steelhead trout parr (Salmo gairdneri) at 125% supersaturation and 10C in water 65 cm deep.

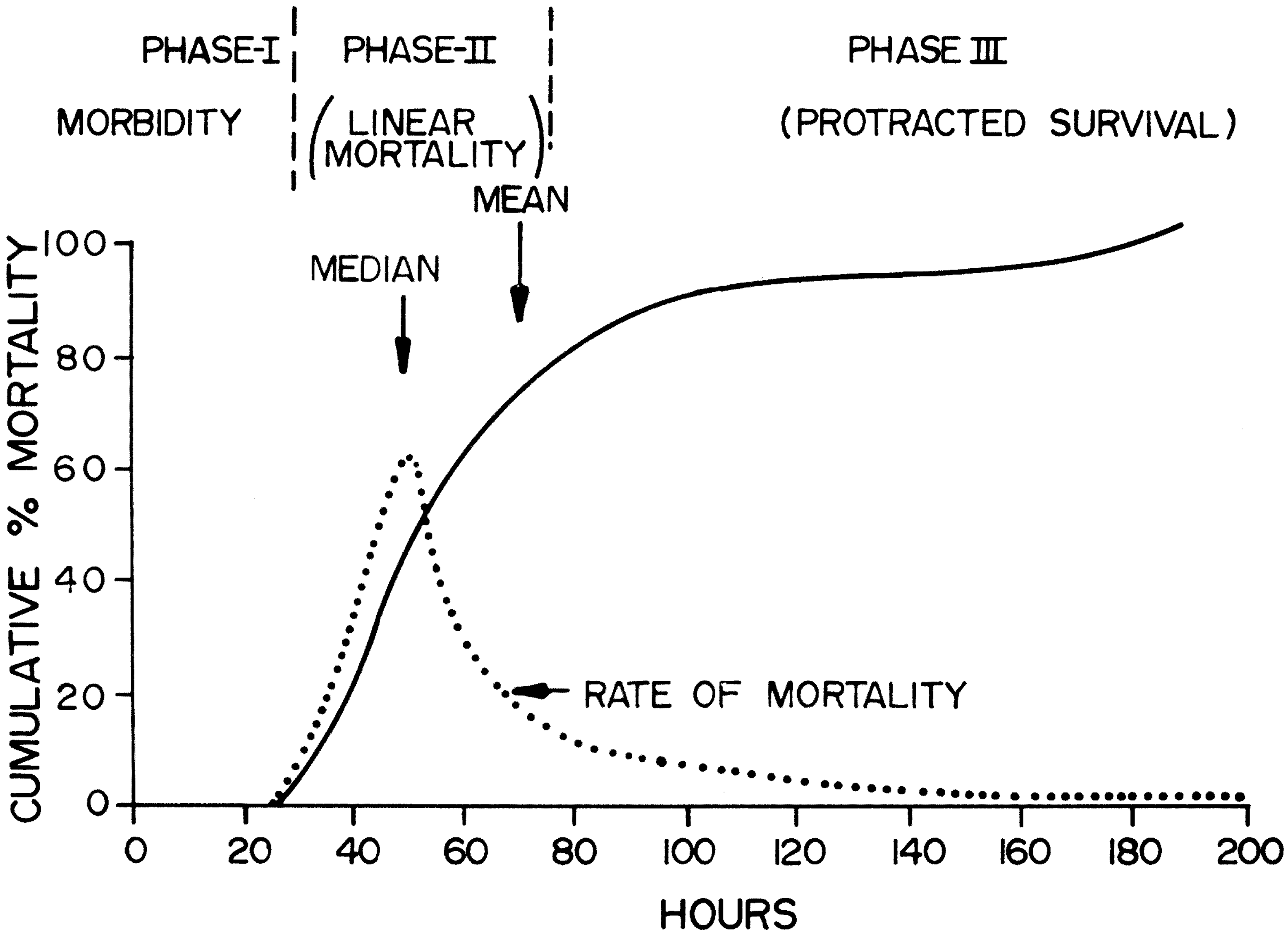


Figure 5. Mortality response of rainbow trout (Salmo gairdneri) to 120% supersaturation.

Skewness was prominent (Table 6) whenever phase III mortality occurred and relatively high whenever the mortality data were plotted as hours to death. Although a log transformation of the data generally diminshed skewness, it still was a prominent factor approaching a high level of statistical significance (Snedecor and Cochran, 1973). Its occurrence at high and low levels of supersaturation indicates that the shortest time to mortality is more sharply defined than the maximum time to death. Further, skewness usually indicates that a relatively small number of factors are influencing the resulting population response, and this may be true for the fish's tolerance to supersaturation. However, skewness varied between tests and, in the case of adult female coho salmon (at 120% supersaturation), the coefficient of skewness increased from 0.235 in late September to 1.195 in late October. Possibly this reflects the impact of selection via prespawning mortality prior to capture but, whatever the cause, it influenced the response curve. Higher levels of skewness were more associated with low levels of saturation, male fish, and immature juvenile fish than their counterparts.

Central tendency was examined by comparing means, median, and modal values. Confidence limits can be derived for means, a valuable advantage when statistically comparing different populations or developing population models. However, mean time to death had required a 100% kill which neither occurred at 115% supersaturation nor, in many cases, at 120% even after prolonged exposure. This was due in part to the skewed population response described above. Thus, the median time to death typically occurred before the more protracted mean time to death. Median time to death, therefore, not only permits more tests to be conducted in any given period, but also is an appropriate measure of central tendency in these tests. However, applying confidence limits to it is difficult.

Table 6 lists mean times to death varied among levels of supersaturation, species, and life stages. The width of the 90% (two-tailed) confidence estimate of the population mean (μ) at 120% saturation varied from a low of about $\pm$ 6 hours for juvenile rainbow trout to a high of $\pm$ 19 hours for adult female steelhead (winter). Confidence belts were generally smaller at higher saturations, and the previous results would predict extremely broad confidence belts at low levels of supersaturation.

Variability was a function, in part, of the supersaturation level, hence coefficients of variation (C.V.) were inversely proportional to the supersaturation level. At 120% saturation C.V. values reached a high of 60. However, variability usually diminished a great deal when the data were expressed at $\log_{10}$ hours to death, and this was the case at each saturation level. This emphasizes the need for a log transformation of exposure times in studying gas bubble disease, as well as toxicants.

Similar to the coefficients of variation, standard deviations were inversely proportional to the saturation level. Frequently the standard deviations approached or exceeded 35% of the means; however, these were much smaller when the data had been transformed into $\log_{10}$ values.

PATHOBIOLOGY AND TIMES TO MORTALITY

Tolerance to 130% Saturation

Spring chinook salmon and largemouth bass were the only species tested at this high level of saturation. The times required to reach 20% mortality and median mortality at 130% are listed in Tables 7 and 8. Adult spring chinook began dying within about seven hours, reaching median mortality in about 8.5 hours and total mortality within 12 hours. From these data continuous exposure to 130% apparently can kill salmonid fish in a relatively short time provided that the fish remains in shallow water. However, if a fish dives to a depth of 3 meters, the hydrostatic pressure of water would prevent bubbles from forming.

Adult spring chinook at 130% of air saturation indicated approaching mortality by their behavior. After 4 hours of exposure, morbid chinook would swim aimlessly, unresponsive to external stimuli. Coughing and shaking occurred frequently, usually followed by bursts of violent but generally aimless swimming. Necropsy found few external signs of gas bubble disease, but emboli usually were present in the blood vessels of the gills causing discoloration. A massive air-embolus filled the intravascular space between the capillary beds of the gills and the heart. Air emboli could be seen in the intercostal arteries and in the dorsal aorta. After median mortality had been reached, small emphysema (bubbles) were evident in some fins of some fish, but usually not seen in the tail of adult spring chinook. Most adult spring chinook salmon sank at death and did not float during the short period between death and necropsy. These results were reported in greater detail by Stroud, Bouck and Nebeker (1975).

Largemouth bass, lethargic at 130% saturation and 10 C, died only after exposures 10 times longer than those lethal to adult spring chinook salmon. The longer survival of bass probably accounts for more fully developed and more frequent incidence of external signs of gas bubble disease in these fish. After two days of exposure, all the bass were still alive, but they contained sufficient emphysema to change portions of their normally transparent fins to a whitish translucent color. Emphysema usually was not observed under the skin or scales of bass. Necropsy revealed that portions of the gills which extended to the heart were filled with air at pressure sufficient to distend the myocardium. Largemouth bass floated prior to and after death from gas bubble disease, possibly because their swim bladder has no opening to their esophagus for volitional release of excess air. This resulted in tympanites of the swim bladder.

TABLE 7. HOURS OF EXPOSURE TO REACH 20% MORTALITY AMONG FISHES EXPOSED TO SUPERSATURATION IN SHALLOW WATER AT 10°C.

	% saturation				
	130	125	120	115	110
Coho salmon					
parr	-	37, 22	30, 73, 134	>336	-
smolts	-	-	-	-	-
held over smolts	-	8, 12	30, 23, 41, 30	120, 303	-
jacks	-	14	49, 40	336	-
adult females (gravid)	-	19	38.5, 43	122	-
adult males (ripe)	-	19	56, 40	207	-
Spring chinook salmon					
parr	-	-	-	-	-
smolts	-	-	30	>268	1440
adult females, migrating	7	14	30, 30	430	-
adult males, migrating	8	15	50, 22	498	-
Sockeye salmon					
parr	-	20	36, 45	49, 167	-
smolts	-	11, 19, 16	35, 27, 50, 58	>268	>268
adults, migrating	-	<18	60	140, 650	-
Steelhead Trout, Summer					
parr	-	-	-	-	-
smolts	-	14, 14, 29	-	>268	>268
adults, migrating	-	25	72	140	-
Steelhead Trout, Winter					
parr	-	19, 38, 20	40, 25	160	-
smolts	-	-	46	154	>268
adult females, (gravid)	-	-	68	332	-
adult males (ripe)	-	-	68	263	-
Rainbow Trout					
parr	-	23, 24	45	-	-
yearlings	-	-	23, 218	79	-

TABLE 7. HOURS OF EXPOSURE TO REACH 20% MORTALITY AMONG FISHES EXPOSED TO SUPERSATURATION IN SHALLOW WATER AT 10°C.

	% saturation				
	130	125	120	115	110
Largemouth bass					
juveniles	115	-0-	-	-	-
adults	65	142	-	-	-
Suckers (*Catostomus* sp.)					
adults	-	-	226	-	-

Tests were conducted with a maximum attainable depth of 65 cm, and 10 fish or more per test.

TABLE 8. ESTIMATED HOURS TO MEDIAN MORTALITY (ET_{50}) AMONG FISHES EXPOSED TO SUPERSATURATION IN SHALLOW WATER AT 10°C.

SPECIES	Gas levels (% saturation) 130	125	120	115	110
Coho salmon (Oncorhynchus kisutch)					
a. parr	---	70, 44	280,120,150	>336	---
b. smolts	---	---	---	>240	---
c. held-over smolts	---	11,13	44,34,40	270,>336	---
d. precocious males (jacks)	---	17	60,60,50	>336	---
e. adult females	---	20	44,52	180	---
f. adult males	---	20	80,71	265	---
Spring chinook salmon (Oncorhynchus tshawytscha)					
a. parr	---	---	---	---	---
b. smolts	---	---	50	>268	>268,>1440
c. adult females, migrating	8.1	17.5	45,60	540	>1440
d. adult males, migrating	8.9	16.0	65,43	500	>1440
Sockeye salmon (Oncorhynchus nerka)					
a. parr	---	40	70,95	100,183	---
b. smolts	---	20,23,22	60,54,60	>268	>268
c. adults	---	<18	83	>168	---
Steelhead Trout, Summer (Salmo gairdneri)					
a. parr	---	19,18	---	---	---
b. smolts	---	---	52	>268	---
c. adults, migrating	---	33	108	>168	---
Steelhead Trout, Winter (Salmo gairdneri)					
a. parr	---	38,70,35	90,75	270	---
b. smolts	---	---	>48	>268	---
c. adult females, gravid	---	---	92	>336	---
d. adult males	---	---	80	>336	---
Rainbow Trout (Salmo gairdneri)					
a. parr	---	25	56	---	---
b. yearlings	---	31	42	140	---
Largemouth Bass (Micropterus salmoides)					
a. juveniles	175	>240	>240	---	---
b. adults	130	>240	>240	---	---
Suckers (Catostomus sp.)					
a. adults	---	---	>216	---	---

Tolerance to 125% Saturation

A 125% saturation was lethal to all the salmonid fish in six days under these test conditions, but largemouth bass and suckers were not killed. Median mortality was reached in an overall average of 28 hours. Time to first mortality ranged from 6 hours for hold-over smolts1/ to 25 hours for adult winter steelhead, but typically occurred after 12 hours of exposure in this shallow water circumstance. Times to median mortality ranged from 11 hours for hold-over coho smolts to 70 hours for juvenile coho salmon and to greater than 240 hours for bass (Table 8). Tolerance differed very little between the sexes of a given species at this level, but different life stages frequently showed marked differences in tolerance, e.g., coho salmon (Table 8). Median survival times were about twice as long for juveniles of winter steelhead as for summer steelhead, indicating that different tolerance may exist due to racial stocks. However, experimental error may account for these differences because variability sometimes exceeded 100%.

At least 90% of the fasting largemouth bass survived 125% saturation for 10 days, but they developed extensive areas of emphysema in their fins. At this time juvenile salmon were added to the tanks, whereupon the bass caught and ate them. This led to the conclusion that the bass were still sufficiently healthy to sustain their predatory inclinations. Meekin and Turner (1974) reported that sublethal gas bubble disease inhibited predation by adult squawfish (*Ptychocheilus oregonensis*).

External signs of gas bubble disease were generally more evident at 125% than at 130% because the fish survived longer and therefore the gas had longer to act upon them. The first mortalities at 125% had minimal external signs of gas bubble disease; typically external signs were more readily apparent on later mortalities. Signs include gills mottled by emboli and emphysema in the fins, along the lateral line, and under the skin. Eye involvement generally was minimal at 125%, possibly because exposure time was inadequate. Steelhead and rainbow trout were the exception and developed both eye lesions and dermal emphysema, which formed sooner and more extensively than among other test fish.

Tolerance to 120% Saturation

Time to median mortality ranged from a low of 34 hours for hold-over coho smolts to as much as 280 hours for coho parr, but no largemouth bass died at 120% saturation. The first recorded mortality to steelhead parr was in 16 hours. Adult salmonid fishes generally survived three to four days before reaching 50 percent mortality.

1/ Smolts not liberated in spring and maintained in tanks until the time of testing in early winter. Hold-over smolts do not exist in nature and generally don't survive.

Differences in tolerance to gas bubble disease were evident at 120% between adult male and female coho; gravid coho females were less tolerant among coho and possibly this also was the case for gravid sockeye salmon. A sex-related difference in tolerance was not noted in adult spring chinook, possibly because they were not gravid. Adult winter steelhead died sooner than adult summer steelhead, again possibly because the winter fish were ready to spawn.

Most of the previously described signs of gas bubble disease were observed at 120% saturation where longer survival, hence longer exposure time, increased the severity of the lesions. Eye damage became evident at this level of supersaturation, but it occurred in probably less than 10% of the fish and primarily among the various racial stocks of Salmo gairdneri. The most frequently observed eye involvement was exophthalmia from retrobulbular emphysema. This caused the eyeball to protrude well beyond the orbit and may have diminished the functional status of the eye. Another form of eye involvement consisted of air bubbles in the aqueous humor and in the retina causing distorted optics, retinal detachment, and hemorrhage. The latter lesions are considered a serious problem that can lead to blindness, inability to spawn, and death.

Another significant result of gas bubble disease was the development of petechial and ecchymotic hemorrhages in various locations including cutaneous and intra-muscular regions. Presumably these hemorrhages occurred in the surviving fish, but their impact and extent are unknown. Also, adult salmon with gas bubble disease frequently developed fungal infections which spread rapidly across their bodies.

Tolerance to 115% Saturation

Prolonged exposure was required to reach median mortality (Table 8) at 115% total gas saturation. Total mortality never occurred at this level during the test period (up to 30 days), hence the average $\log_{10}$ time to death could not be computed. The most sensitive fishes were sockeye parr (ET_{50} in 100 hours) and adult female coho (ET_{50} in 180 hours), and the least sensitive salmonids were juvenile coho salmon (0 mortality in 336 hours). Intermediate tolerance was indicated by juvenile steelhead (ET_{50} in 270 hours) and adult spring chinook (ET_{50} in 526 hours). Sex related differences were more pronounced at this level.

Symptoms of gas bubble disease were most developed at 115% mainly due to the prolonged exposure to supersaturation. Exophthalmia became even more exaggerated and frequent but still involved probably less than 10% of the animals. Emphysema were abundant in the skin and fins of essentially all the fish. Fungal infections became very frequent and covered areas of ulceration. Petechial hemorrhage of hyperemia often gave the skin a reddish color.

Muscular emphysema were noticed in adult spring chinook after two weeks of exposure at 115%. The emphysema grew in size as exposure time increased to three weeks until slices of the muscle were so riddled with holes that they resembled red "swiss cheese." The size of these emphysema, described in greater detail by Stroud and Nebeker (1975), reached to slightly greater than 1 cm in diameter.

Tolerance to 110% Saturation

Only a few tests were conducted at 110% total gas saturation and were generally tolerated by the test fish. One chronic exposure was conducted on spring chinook salmon adults and smolts. During a 3.5 month period, 10% of the adults died of gas bubble disease and secondary complications. Similarly, 20% of the spring chinook salmon smolts died in two months at 110%. In this case about 30% of the smolts had exophthalmia and at least 50% had other external signs of gas bubble disease such as emphysema in the skin or fins. Perhaps emphysema would have been discovered in even more fish had we inspected them via ultrasonic sound reflection (Mackay and Rubissow, 1971). Necropsy revealed that most of the dead smolts had emboli in the blood.

Food pellets were offered to the spring chinook smolts to prevent starvation during the long exposure period. Those smolts with exophthalmia attempted to and could usually feed, but their actions suggested impaired vision and, in some cases, blindness.

ESTIMATED TIMES TO 20% MORTALITY (THRESHOLD TOLERANCE)

We established the time to 20% mortality as the threshold for mortality. Based on the previously described skewed mortality curves, additional exposure will probably produce disproportionally greater mortality when the 20% mortality level is achieved. Conversely, remaining well below this exposure time would probably prevent losses due to gas bubble disease. Therefore we estimated the true population mean (μ) from the observed times to 20% mortality (x) by determining the 90% (two-tailed) confidence limits of the sample mean ($\bar{x}$). Although the curves are skewed, the data were pooled from all salmonid species of a given life stage because we have not yet proven that these species were significantly different, especially in practical terms.

The 90% confidence limits for average time to 20% mortality are listed in Table 9 and shown in Figure 6. At the highest stress level (125% saturation), one can readily predict within narrow limits the probable time to 20% mortality for a given life stage. Smolts and adults required about 3/4 of a day and parr required nearly a full day at 125% saturation to reach 20% mortality. As the stress level diminished to 120% of saturation, time to 20% mortality approached two days of exposure

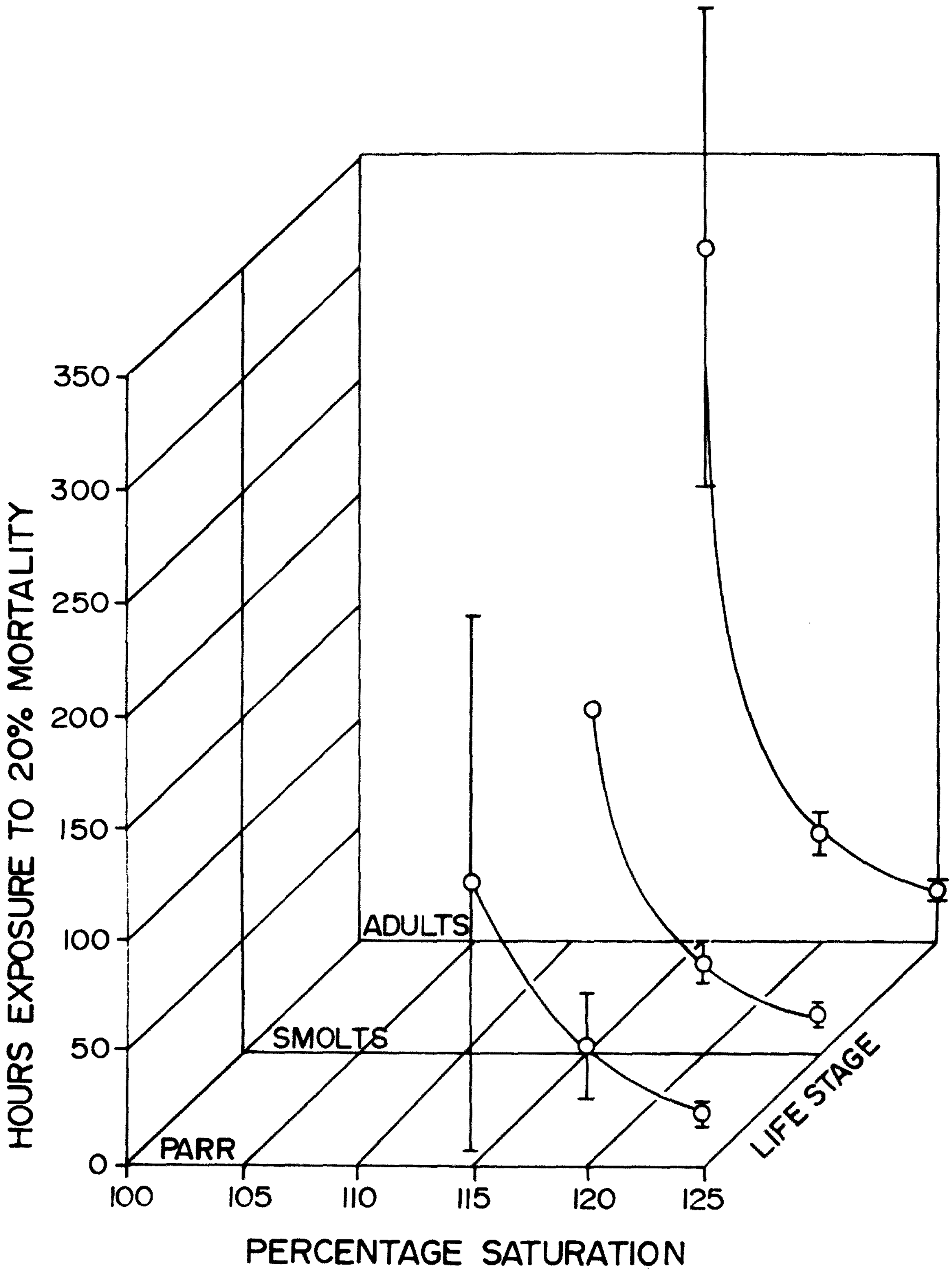


Figure 6. Means and 90% confidence belts of times to 20% mortality at various life stages of salmonids and levels of supersaturation.

TABLE 9. STATISTICAL ANALYSIS OF POOLED OBSERVATIONS ON HOURS TO 20% MORTALITY AMONG THREE LIFE STAGES OF FIVE SPECIES OF SALMONIDS.1/

	LIFE STAGE		
	Adults	Smolts	Parr
125%			
mean hours	18.33	17.17	23.63
90% limits of mean	±3.19	±5.24	±4.41
range	14-25	11-29	14-38
n	6	6	11
120%			
mean hours	48.12	41.00	53.50
90% limits of mean	±8.69	±10.06	±23.83
range	22-72	27-58	25-134
n	12	6	8
115%			
mean hours	309.11	154	125.30
90% limits of mean	±114.28	-	±122.25
range	122-650	-	49-67
n	9	1	3

1/ Included spring chinook salmon, coho salmon, sockeye salmon, summer steelhead trout, winter steelhead trout, and rainbow trout.

and the confidence belt ranged in width from 8 hours to 24 hours. At the lowest stress level that routinely produced mortality (115% saturation), mean times to 20% mortality increased to about 13 days (± 5 days) of exposure for adults and about 5 days (± 5 days). Stress levels at 110% saturation did not routinely produce mortality.

Salmonid fish must typically spend several days at a maximum depth of less than 60 cm if their populations are to experience as much as 20% mortality at saturation levels of 115%. However, caution is urged because the confidence limits are approximately 100% of the mean, hence the response is relatively unpredictable at low stress levels. Evidently several factors influence tolerance at low levels of saturation, and some of these are indicated in the sub-section on factors affecting mortality by supersaturation.

RANK ORDER OF TOLERANCE TO SUPERSATURATION

The life stages of the various test species were placed in a rank order beginning with the least tolerant and proceeding to the most tolerant (Table 10). The data base was constructed from the estimated time to median mortality at 120% total gas saturation (203 g/cm^2 hyperbaric). The authors selected this saturation level because the resulting mortality was sufficiently protracted to permit the expression of inherent differences while generally excluding the effects of disease and malnutrition

FACTORS AFFECTING MORTALITY BY SUPERSATURATION (GAS BUBBLE DISEASE)

Test Temperature

Mortality at comparable saturation levels was determined at temperatures between 10 C and 20 C. Sockeye salmon and largemouth bass (both adults and juveniles) reveal markedly different response patterns to supersaturation when tested at different acclimation temperatures (Figure 7 and Table 11). Adult sockeye were considerably more tolerant to 120% and 125% saturations when they were acclimated slowly from 10 C and tested at 18 C. But sockeye salmon and rainbow trout parr showed a variable response; increased test temperatures increased tolerance in one case and decreased tolerance in two cases. Both juvenile and adult largemouth bass showed a decrease in tolerance with increased test temperature, possibly because they were stressed by temperature acclimation and by supersaturation.

Obviously, test temperature has an influence on tolerance to supersaturation, but resulting variability creates uncertainty in interpreting the impact of acclimation time, temperature preference, or both. For example, adult sockeye had been acclimated to their

TABLE 10. RANK ORDER OF TOLERANCE BETWEEN FISH TESTED AT 120% TOTAL GAS SATURATION (203 g/cm^2 hyperbaric). 1/

Species	ET_{50} 2/	Tolerance ratio 3/
Held-over coho smolts	39.3	1.00
Yearling Rainbow Trout	42.0	1.06
Adult female coho	48.0	1.22
Spring chinook smolts	50.0	1.27
Summer steelhead smolts	52.0	1.32
Adult female spring chinook	52.5	1.34
Adult male spring chinook	54.0	1.37
Rainbow trout parr	56.0	1.42
Precocious male coho (jacks)	56.6	1.44
Sockeye smolts	58.0	1.48
Adult male coho	75.0	1.90
Adult male winter steelhead	80.0	2.04
Sockeye parr	82.5	2.10
Winter steelhead parr	82.5	2.10
Adult sockeye	83.0	2.11
Adult female steelhead	92.0	2.34
Adult summer steelhead	108.0	2.75
Coho parr	183.0	4.66
Largemouth bass	>240.0	>6.00

1/ Tests were conducted at 10°C with a maximum water depth of 65cm.

2/ Times to median mortality averaged from Table 8.

3/ Tolerance ratio = $\frac{\text{"x" time to median mortality}}{\text{smallest time to median mortality}}$

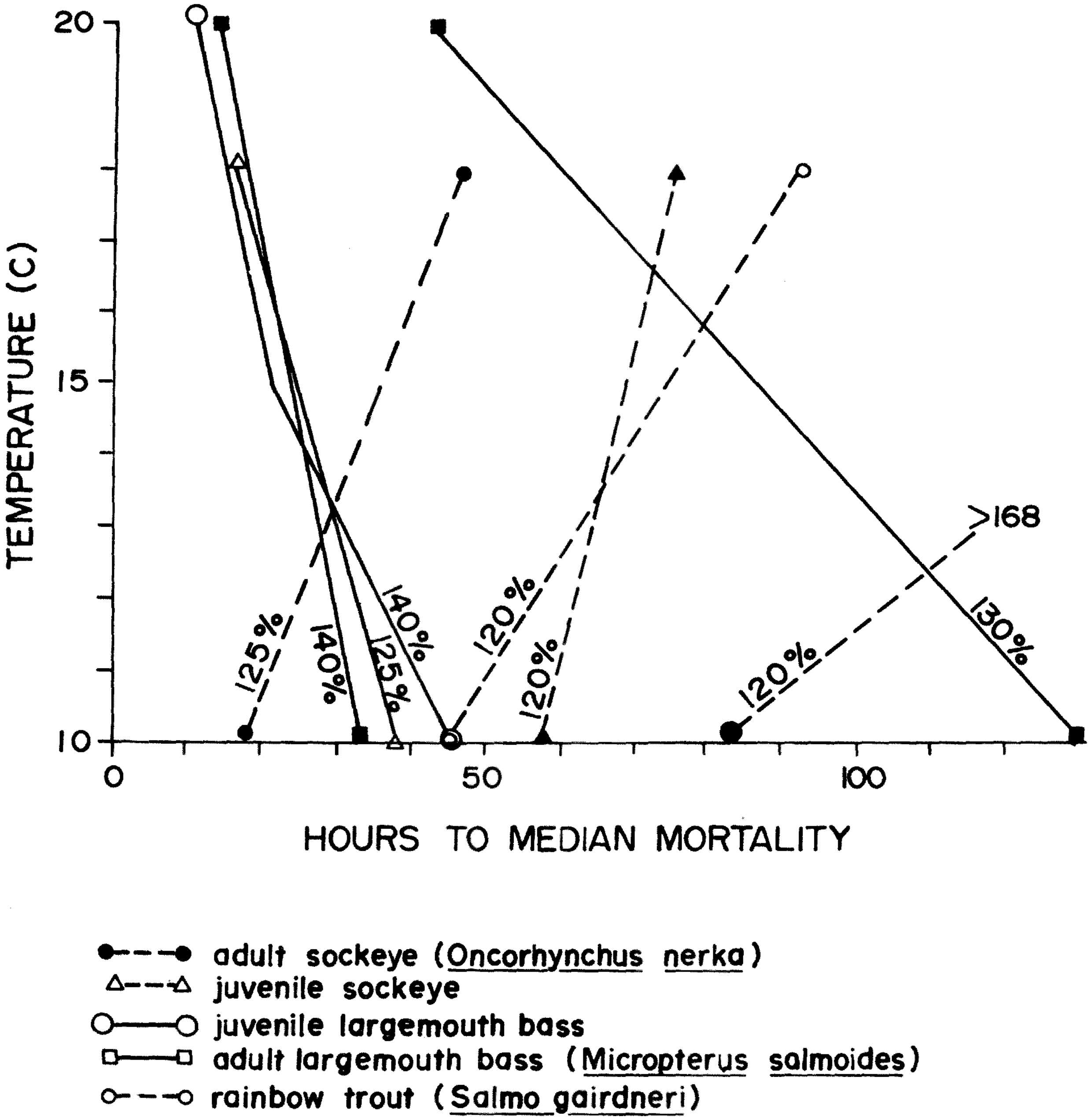


Figure 7. Effects of test temperature on the time to median mortality from supersaturation.

TABLE 11. SUMMARY OF THE RELATIONSHIP BETWEEN ACCLIMATION TEMPERATURE AND TIME TO MEDIAN MORTALITY IN SUPERSATURATED WATERS.

Species and Saturation level	Hours to median mortality at temperature		
	10 C	15 C	18 C
1. Sockeye salmon (*Oncorhynchus nerka*)			
a. adults (1) 125%	18		46
(2) 120%	83		168
b. parr (1) 125%	38		16
(2) 120%	58		75
2. Rainbow trout (*Salmo gairdneri*)			
a. parr 125%	29		18
120%	46		92
3. Largemouth bass (*Micropterus salmoides*)			
a. adults (1) 140%	32	22	12
(2) 130%	130		42
b. juveniles (1) 140%	45	21	10
(2) 130%	175		60

test temperatures for about three weeks prior to testing. These fish showed increased tolerance to supersaturation at higher acclimation temperatures. This was also the case for one group of juvenile sockeye and rainbow trout which were well acclimated to warm water. All of the other salmonid fish in the experiment had little or no time for temperature acclimation (juvenile sockeye and rainbow trout) and they showed decreased tolerance to supersaturation.

Bass had been collected by seining farm ponds on November 27, 1972, and some fish were tested at 10 C beginning December 4, 1972. The bass were kept at about 10 C until March 19, 1973. Then the fish were warmed to 20 C in about 4 days and allowed to adjust for an additional 2.5 days prior to beginning the test. This procedure was repeated several times in the testing of the bass.

Whether the relatively rapid change from cold to warm temperatures had influenced the tolerance of bass is unknown, but their response conforms to the pattern established by salmonids which experienced minimal temperature acclimation time. However, bass prefer warm water and were more active at 20 C than 10 C. Possibly the observed differences in tolerance to gas bubble disease between bass and salmon are based on more fundamental differences between warm water fishes and cold water fishes.

Based upon these data, it is obvious that temperature can influence tolerance to supersaturation. Unfortunately more work will be needed to clarify the role of temperature.

Swimming Activity

The effects of swimming activity were studied on juvenile sockeye salmon and bass in supersaturated water; results for largemouth bass are shown in Figure 8. Bass kept in cages in the tank center experienced very little current or need to swim and 50% survived for 26 hours. But bass that were placed concurrently into the outside portion of the tank (higher velocity) took 25% less time to reach median mortality. Juvenile sockeye died at about the same rate regardless of their location in the tank. Increased mortality resulting from activity was seen in other tests. Those individuals which were extremely active and often jumped out of the water usually died before those which remained relatively quiet.

Sex

In some circumstances differences in tolerance to supersaturation varied between sexes for adult fish (Figure 9). Tolerance was different between males and females when (1) the rate of mortality was low enough

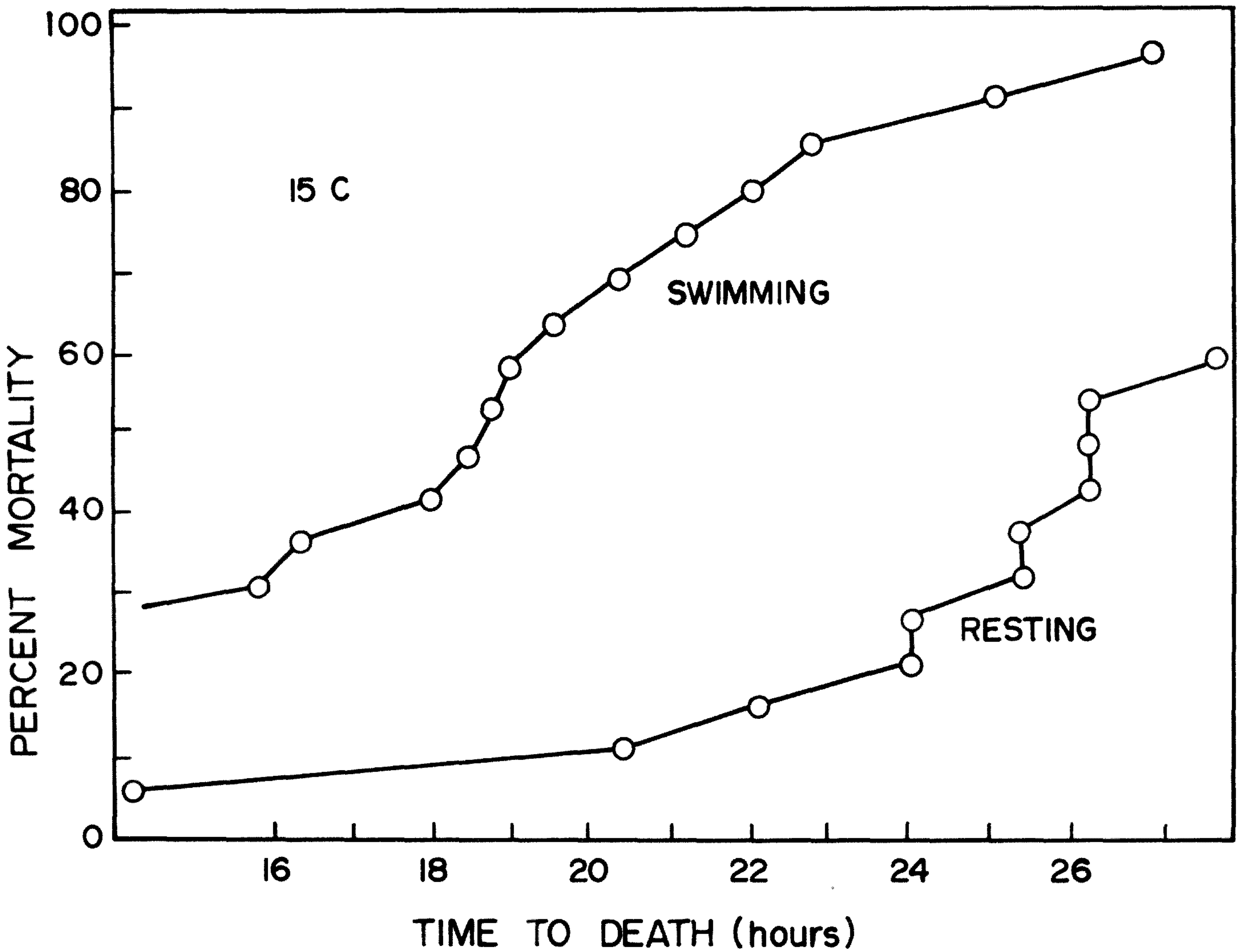


Figure 8. Effects of swimming activity on the mortality of largemouth bass (Micropterus salmoides) in water supersaturated to 140%.

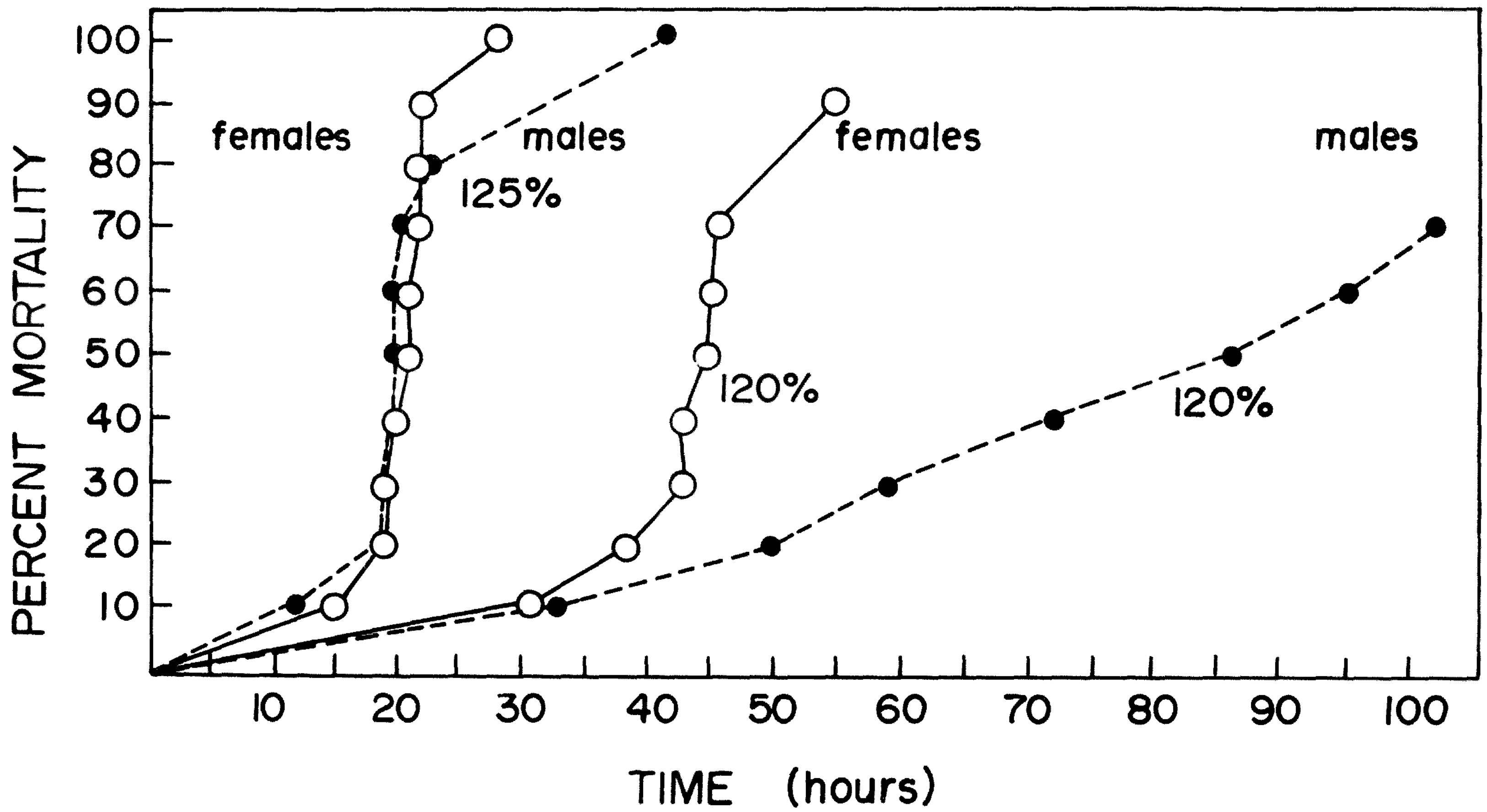


Figure 9. Effect of percent saturation and sex on mortality of adult coho salmon (*Oncorhynchus kisutch*) at 10C in tanks 65 cm deep.

to permit its expression, and (2) if significant collateral differences also existed. For example, female and male salmonids both died very rapidly at or above saturation levels of 125% and there was no apparent difference in tolerance. Also, no apparent difference was evident at 120% or lower when male and female fish experienced comparable conditions, i.e. migrating spring chinook salmon. However, when the males and females were not in comparable condition, the result was a difference in tolerance to supersaturation. For example, male and female winter steelhead showed a difference in tolerance, but this was probably due to fighting among the males which weakened them. Female coho salmon were less tolerant than males and we speculate that this difference was due to the weakened condition of the gravid females (Figure 10).

Largemouth bass did not die except at high levels of supersaturation which may have precluded the expression of sexual differences. No differences were noted in the rate or total mortality between male and female largemouth bass tested at 130% saturation and 20 C.

Life Stage

Figure 10 shows the concurrent times to mortality among juveniles (parr), post smolts, jacks and adult coho salmon. Median mortality was reached first by adults, then by jacks, and last by parr at 120% saturation. At 115% saturation proportionally fewer adults were killed and essentially no juveniles were killed.

Body Size and Condition Coefficient

Body size and/or condition had a significant influence on time to death in some instances. Correlation analyses were conducted for $\log_{10}$ hours to death on body weight, body length, and condition coefficient (K) for adult coho. The only significant correlation at 125% saturation was between the length of female coho and $\log_{10}$ hours to death ($r = 0.93$); larger fish took longer to die. These fish were essentially gravid and ready to spawn. Juveniles were not tested.

Adult spring chinook had a significant correlation between $\log_{10}$ time to death and body weight (Table 12). While these results have statictical significance and indicate that larger adult salmon tend to survive longer in supersaturated water, the overall impact seems relatively small and questionable. For example, Meekin and Turner (1974) determined that the size of juvenile Pacific salmon was inversely related to survival in gas supersaturated water; large juveniles died sooner than their smaller siblings. Perhaps fat content influences this as indicated by Boycott and Damant (1908) and Gersh, Hawkinson, and Rathbun (1944).

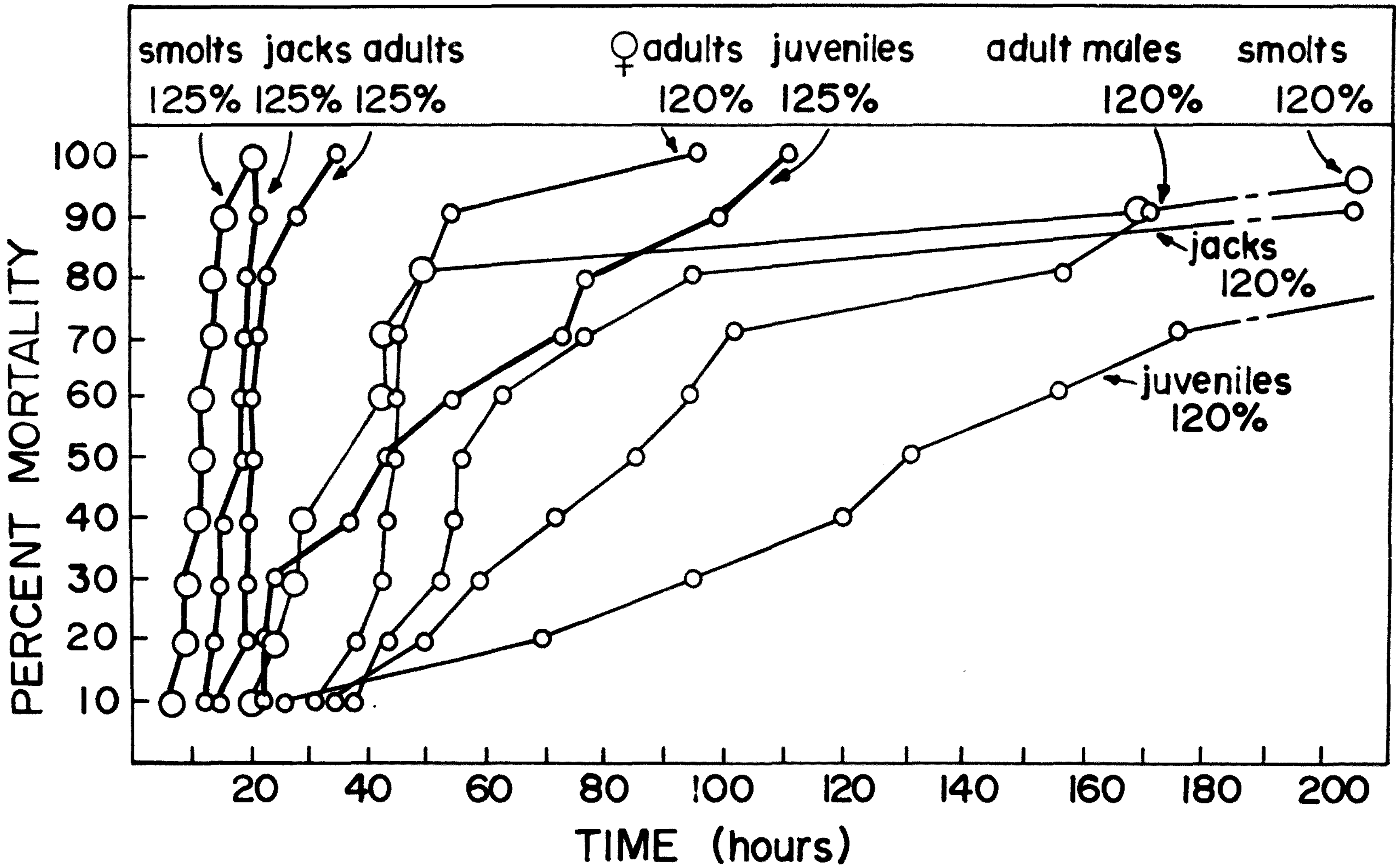


Figure 10. Effect of percent saturation and life stage on mortality of coho salmon (Oncorhynchus kisutch) at 10C tanks 65 cm deep.

TABLE 12. CORRELATION ANALYSES OF TIME TO DEATH AT VARIOUS LEVELS OF SUPERSATURATION TO BODY LENGTH, WEIGHT, AND CONDITION COEFFICIENT (K) FOR ADULT SPRING CHINOOK (*Oncorhynchus tshawytscha*).

Total Gas Saturation	Sex		Body Length (cm)	Body Weight (g)	Condition Coefficient (K)
130%	Males (9)	r =	+0.653*	+0.679*	+0.278
		a =	-41.18	-22117	+1.163
		b =	+121.74	+29370	+0.017
	Females (11)	r =	+0.480	+0.349	-0.307
		a =	+15.823	-6056	+1.672
		b =	+70.628	+14928	-0.036
125%	Males (5)	r =	-0.674	-0.820	+0.946*
		a =	+87.384	+4563	-3.745
		b =	-13.838	-1184	+0.007
	Females (15)	r =	+0.225	+0.137	-0.167
		a =	+37.143	+1221.	+1.207
		b =	+34.320	+13593	-0.009
120%	Males (9)	r =	+0.978*	+0.813*	-0.690*
		a =	-130.82	-12818	+5.813
		b =	111.92	10825	-2.416
	Females (8)	r =	+0.970*	+.723*	+0.688*
		a =	-90.52	-3294	+1.137
		b =	81.03	+6267	+0.085
120%	Males (7)	r =	-0.642	-0.624	+0.047
		a =	+177.9	25421	+1.236
		b =	-61.09	-17713	+0.059
	Females (9)	r =	-0.650*	+0.659*	-0.252
		a =	-12.132	-14240	+1.568
		b =	+54.793	+12712	-0.152
115%	Females (7)	r =	+0.837	+0.487	+0.520

* Significant at 90% level.

Other Factors Influencing Tolerance to Supersaturation

At least two other factors may influence tolerance to gas bubble disease based on our observations. The first is the effect of small but definite increases in dissolved gas pressure induced by solar radiation and fluctuations in the control system. Slight increases in saturation seemed to cause increases in mortality in critically morbid fish; conversely, small decreases in saturation caused decreases in mortality. Possibly the same effect was caused by changes in depth which would alter the amount of hydrostatic pressure to compensate the emboli or emphysema.

A second important factor that is difficult to quantitate is behavior. The investigators spent many hours watching the test animals and agree that behavior can have significant impact upon the results. For example, adult sockeye salmon are schooling fish and tend to pursue a common mode or level of activity. Other fish such as adult steelhead tend to be very individualistic and their results seem to be more variable. Adult chinook tended to remain at maximum depth in the tanks; if this is the case in nature, the investigators speculate that these fish would probably find ample compensation by hydrostatic pressure, if available.

TOLERANCE TO SEAWATER AFTER EXPOSURE TO SUPERSATURATED WATER

Three species of anadromous salmonids were tested for tolerance to seawater (30 ppt Cl) after exposure to three levels of supersaturation (110, 115, and 120%).

Exposure to 120% continued for about 47 hours when the following levels of mortality had been reached: winter steelhead = 15%; sockeye salmon = 45%; spring chinook salmon = 60%; and summer steelhead = 70% (Figure 11). Immediately thereafter the fish were transferred to air equilibrated seawater and no further mortality occurred during a subsequent 96 hour period that could be related to supersaturation or seawater.

Other groups of smolts were placed in 115% saturation water for 268 hours. During this time 10% of the sockeye smolts and 20% of the winter steelhead smolts died of gas bubble disease. None of the summer steelhead or spring chinook smolts died during the 268 hour exposure to 115% saturation. However, the survivors of each species had some emphysema in the skin and fins and a few had protruding eyes from emphysema around or behind the tye. Signs of gas bubble disease were most prevalent and severe among the winter and summer steelhead. Yet these fish survived direct transfer to 30 $^{0}/_{00}$ seawater and continued to survive for 124 hours when the experiment was ended.

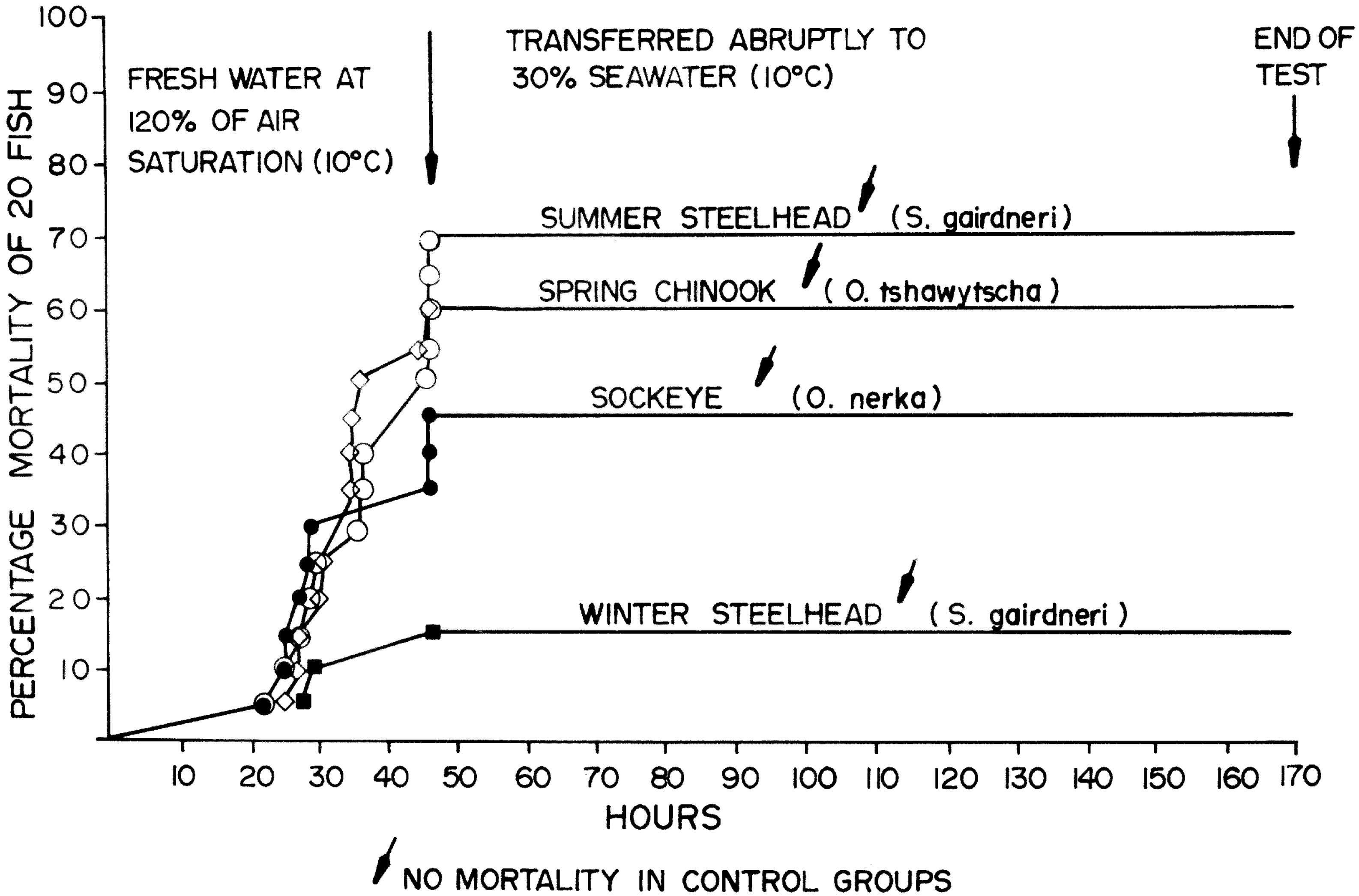


Figure 11. Cumulative mortality of salmonid smolts during exposure to 120% air supersaturation and immediate transition to $30^{0}/oo$ seawater.

No smolts died that were exposed to 110% saturation for 268 hours followed by abrupt transfer to 30 o/oo seawater.

These results confirm our previous pilot studies on coho salmon smolts and indicate no latent mortality to smolts from gas bubble disease after they have entered seawater. Essentially identical results were reported by Dawley, Monk, Schiewe, and Ossiander (1975).

EFFECTS OF CHRONIC SUPERSATURATION ON REPRODUCTION

The effects of supersaturation on reproduction were investigated only at 110% saturation because higher levels of supersaturation produced extensive mortality of adults in shallow tanks. The exposure of 20 adult spring chinook began April 30, 1973. One female developed partial paralysis early in July and was sacrificed and examined about three weeks later. Another fish died at about this time when dissolved gas levels jumped unexpectedly to 116%, but this fish had a heavy infection in the gills by fungus and parasitic copepods. The test exposure ended August 3 when it became apparent that further mortality would result if we did not treat for the infective fungus and copepods. Thereafter no mortality occurred in either group until spawning operations began. Apparently the infections had weakened both the control and test fish to the extent that heavy mortality occurred each time the fish were handled and inspected for sexual maturity.

Experimental male spring chinook salmon ripened readily and their semen was normal in appearance and consistency. Likewise, the eggs of all experimental females developed to typical size and were normal in color and general appearnace. However, prespawning mortality from handling the fish eliminated four of the six females in the experimental lot; extensive mortality also occurred in the control lot.

Two experimental females survived the stress of supersaturation and subsequent prespawning handling; these ripened and were spawned artificially. Their eggs were typical in all respects and approximately 90% of these eggs hatched. This is well within normal limits and was comparable to the control fish. The results of this limited sampling revealed no evidence of adverse effects to the sex products or to the ability of the resulting embryos to hatch.

SECTION V

DISCUSSION

Perhaps some readers may want to extrapolate beyond these data to natural circumstances in a river, reservoir, or lake. We urge extreme caution in such endeavors because the research design did not permit the testing of remedial behavioral responses such as avoidance, sounding, or other aspects resulting in less than continuous exposure to supersaturation stress.

Behavioral responses are important because they are known to influence survival in supersaturated water. Dawley, et al., (1975), reported that juvenile fall chinook sustained less than 5% mortality after 60 days of exposure at 120% supersaturation when a maximum depth of 2.5 m was permitted; greater than 95% mortality occurred in the same period when siblings were exposed to 120% supersaturation in water 0.25 m deep. Meekin and Turner (1974) reported similar results for juvenile fall chinook salmon, juvenile coho salmon, and squawfish, but juvenile steelhead trout did not survive quite as well.

In 5-hour tests Meekin and Turner (1974) reported that juvenile chinook salmon avoided supersaturated water and congregated in the control water. Juvenile coho salmon showed no preference during this short period, which may in part, be related to their great tolerance for supersaturation. Juvenile sockeye salmon consistently sound to maximum depth when placed in supersaturated water hence actively avoid gas bubble disease (Wesley J. Ebel, personal communication). Thus avoidance responses could exert an extremely important influence upon the potential lethality of supersaturation in a river.

Tolerance to gas bubble disease appears to involve different biological factors at high versus low levels of supersaturation. In acutely lethal conditions, emboli form in the blood and cause rapid death. Thus survival at acutely lethal conditions may be influenced heavily by the ability of individual fish to tolerate changes in vascular dynamics. But survival at long term sub-acute exposures may be more dependent upon complex alterations of physiological functions such as immune responses, infectious agents, or adaptive behavior.

Several other factors were identified that influenced tolerance to supersaturation, including temperature, activity, life stage, and body size. One of the most important was the difference in tolerance between fish families, and to a lesser extent, tolerance varied between species or races. Another obvious difference was that trout and salmon which are phyostomous were generally more sensitive at 10 C than bass which are physoclistous. One hypothesis to explain this difference is based on the observation that adult salmon expell gas frequently from their swimbladder and sink when dead. Conversely, bass became bloated with considerable pressure in their gas bladder and usually floated before death. Possibly the tolerance of bass to supersaturation was promoted in part, by a concurrent and compensatory increase of intrabody pressure. Evidence of high intrabody pressure was indicated in necropsy examinations. When punctured, the heart and swimbladder emitted an audible release. High intrabody pressure would keep gases in solution within vital organs of the area adjacent to the body cavity and thus protect the fish. However, a sudden release of gas (from the swimbladder of salmon) would tend to sharply diminish intrabody pressure and promote cavitation of gas or the growth of accumulated emboli.

Apparent differences in tolerance to supersaturation were related to genetic stocks, sex, and life stage, but the attendant variation renders it impossible to determine appropriate significance. Sex was a factor in two cases, but only when the respective species approached spawning condition. Adult male winter steelhead were in spawning condition when tested and fought territorial battles in the tanks which may have promoted emboli formation and death or decreased resistance. Whatever the reasons, male steelhead died much sooner than the females. Adult summer steelhead in spawning condition did not fight and, although the sample size was small, there was no readily apparent difference in tolerance between males and females. The other case of sex-related tolerance occurred with adult coho salmon, where gravid females died more rapidly than ripe males. Although the evidence is not clear, indications are that poor physiological condition is a greater influence on tolerance than sex *per se*. Physiological condition may well be the cause of observed differences in tolerance to supersaturation between different life stages.

Osmotic and ionic regulation in seawater provides great stress to smolts of Pacific salmon and steelhead. If these functions are impaired to any great extent, the fish generally die within a few days. In this study the survivors of lethal supersaturation apparently suffered no significant impairment of osmotic and ionic regulation because they survived direct transfer to seawater (30 °/oo) and no mortality resulted within a five-day period thereafter. This result was confirmed by Dawley, *et al*., (1975), and by Bouck[1/].

Gas composition in these experiments was not determined beyond the oxygen level which was typically high. High oxygen-nitrogen ratios have been shown by Rucker (1974) to prolong survival. Apparently helium

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1/ Bouck, G. R. 1972. Unpublished data on coho salmon smolts.

has a similar effect because the decompression period of skin divers can be reduced 50% when half of the nitrogen in the breathing air is replaced with helium (Workman, 1963). Gas composition was not controlled in this experiment and its variability may have influenced the resulting times to death.

Residual effects on sex products were not readily apparent among Columbia River spring chinook salmon which had been held for 3.5 months in shallow water saturated to 110% of barometric pressure. This is more than twice the exposure period that these fish would experience in passing through the Columbia River. The resulting hyperbaric pressure is approximately equivalent to what a chinook would experience at a depth of about 2 meters in water with 130% saturation. Natural spawning was not possible in this research, but the sex products had a good appearance when spawned artificially, and both fertilization and hatching were within normal limits.

Variability was relatively large for the observed times to median mortality; this was true even within a life stage of a given race of a given species. The ET_{50} value varied by 100% in some cases. If one adds to this the potential influence of depth, swimming activity, and acclimation temperature, it is then easy to hypothesize that data from most salmonids can be treated as if they were from the same population without regard to race or species. Thus it appears that the sum of the factors causing increased or decreased tolerance to supersaturation tend to overwhelm the influence of a single factor except when it achieves critical importance on a given occasion.

The interpretation of these data relative to the Columbia and Snake Rivers requires the consideration of many factors. Wild fish live in a dynamic circumstance and are free to alter their depth which, in turn, alters their compensation by hydrostatic pressure. Likewise, in nature intermittent exposure is probably the rule thus permitting higher tolerance than indicated by these data. Also, the potential exposure time of fish must be considered. For adult salmon in the Columbia River typical passage time between the ocean and safe tributary streams is probably three weeks or less.

There is no question that the three species of Pacific salmon, racial variants of steelhead trout, and even bass, can get into serious trouble if they remain in shallow water at high saturation levels for over a day. This has considerable significance in fish hatcheries and laboratories where exposure to supersaturation may be for a longer period and in shallow water. What must be determined is the extent that these data apply to a large and complex river ecosystem.

Supersaturation appears to be exerting ecological selection pressure in the Columbia River Basin (Ebel, et al., 1974), but its direction and final result are not evident yet. Thus far, most researchers have investigated only the detrimental effects of supersaturation, describing supersaturation only in terms of morbidity and mortality. But broader ecological effects are potentially possible and remain undescribed. For example, a low level of supersaturation (or intense supersaturation at certain times of the year) might be an effective tool in eradicating rough fish populations. From this aspect supersaturation has the distinct advantage of leaving no residue, harmful or otherwise. Likewise, if supersaturation kills bass fry and inhibits predation by squawfish, the net result could benefit salmon. Further, if supersaturation selectively eliminates small adults from breeding populations, the net result might be larger salmon therefore better adapted to the Columbia River. This is also suggested by the studies of Cramer (1974).

While a supersaturation level of 110% was not acutely lethal, it did produce low level mortality and signs of ill health in adult and juvenile spring chinook salmon during a 3.5 month laboratory exposure in shallow water conditions. Such a prolonged exposure (especially in shallow water) would be unlikely for wild chinook, but may occur in fish culture stations or possibly in the outfalls of heated effluents. The latter could be particularly important when seasonal temperature cycles dropped beyond the preferred temperatures of local fish species. In such cases, fish congregating in the warmer water might die or be adversely affected by gas bubble disease. Therefore, a saturation level of 110% might be acceptable for a river, but may not be acceptable for heated effluents.

SECTION VI

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16. ABSTRACT

Tests were conducted using continuous exposure in shallow water at levels of total dissolved gas pressure ranging from 110-140% of barometric pressure (hyperbaric pressure = 103-410 g/cm^2). Both times to 20% and to median mortality were determined on several life stages of Pacific salmonids (Oncorhynchus and Salmo) and Largemouth bass (Micropterus salmoides). Mean times to 20% mortality at 115% total gas saturation were 309, 154 and 125 hours for adults, smolts and parr. At 120% saturation mean times to 20% mortality were 48, 41 and 53 hours for adults, smolts and parr. At 125 % saturation, mean times to 20% mortality decreased to 18, 17, and 24 hours for adults, smolts, and parr. Factors which influenced time to death included genera, life stage, acclimation temperature, activity level, sex and body size. Mortality curves were typically skewed to the right. Gross pathology of gas bubble disease was described relative to these experiments. High gas levels that killed 50% of three species of salmon smolts had no apparent effect on the ability of the survivors to tolerate an immediate transfer into seawater (30 ppt Cl). Long-term (3-month) continuous exposure of adult spring chinook salmon to 110% saturation had no readily apparent adverse impact on the fertilization and hatching of their eggs.

17. KEY WORDS AND DOCUMENT ANALYSIS

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