

MASTER

EFFECT OF THERMAL HISTORY ON THE RESISTANCE OF COLUMBIA
RIVER STEELHEAD TROUT (SALMO GAIRDNERI) TO THERMAL STRESS

by

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INTRODUCTION

One of the most valued salmonids inhabiting the waters of the Pacific Northwest is the steelhead trout (Salmo gairdneri). Held in the highest regard by the sports fishermen, the steelhead is perhaps one of the most avidly sought fish of the area. Over the years, however, the numbers of steelhead occurring in the rivers of the northwest have had to be augmented by hatchery reared fish. The construction of hydroelectric dams on the Snake and Columbia Rivers have severely hampered the natural spawning of the species. Each dam that was installed created vast reservoirs of slow moving waters which inundated the gravel beds required by the fish for spawning. The reduction in spawning sites resulted in reduced numbers of native steelhead. A second, but no less important result of the dams has caused further serious loss of steelhead and other species. This second loss is due to gas supersaturated water created by entrainment of air as water passes over dam spillways. This occurs in the spring and early summer when the steelhead are migrating downstream and large numbers of fish are lost to gas bubble disease (Ebel, 1970).

To counterbalance the loss of these fish the states of Washington and Oregon have gone to extensive hatchery rearing of fish which are then released in the rivers. Washington State has fourteen hatcheries and ten semi-natural rearing ponds used in steelhead production. The production of these facilities in 1970 resulted in the release of 5.3 million steelhead (Ayerst, 1971). The smolt-sized steelhead are released at multiple sites along the Columbia River in April and May each year. The size of the fish at release time is approximately ten

fish per pound. Until recently, two years were required to raise steelhead to this size. Advancements in artificial diets have reduced rearing time to one year.

With the release stocks of fish originating from twenty-four different sites spread out over the state it is obvious that a complexity of thermal histories is represented. Water supply for these hatcheries and ponds is taken from a variety of rivers, streams, and wells. The range in rearing temperatures can be considerable. In some cases fish are incubated in a hatchery and then the fry are moved to one of the semi-natural rearing ponds for a period of time prior to release. The point here is that stocks from different hatcheries can represent different long term thermal histories. Previous thermal experience is vastly important to the ability of a fish to cope with thermal stress (Brett, 1952; Hart, 1947; Morris, 1962). If fish stocks should meet a thermal challenge as they pass downstream, e.g., a nuclear reactor outfall, thermal history may play an important role in their survival. Steelhead stocks from a hatchery with water slightly warmer than a second hatchery may be significantly more resistant to high temperature stress. It is the purpose of this study to evaluate the effect of long term thermal history on the resistance of steelhead trout to thermal stress.

MATERIALS AND METHODS

The steelhead trout used in this experiment were two years old at the time the thermal tests were performed. The stocks were obtained from the Naches, Washington hatchery as fry in Spring 1969. They were transported to the Pacific Northwest Laboratory at Richland, Washington where they were divided into four equal groups. The young fish were reared with one of the four groups at each of the following temperatures: ambient Columbia River temperature, 1.7° C, 2.2° C, and

2.6° C above the ambient river temperature. These thermal increments were accurately maintained over the seasonal fluctuations of the river water temperature. The methods used in handling, incubation, and maintenance were all according to normal hatchery operation routines.

After maintenance for two years on the described thermal regime the fish were prepared for thermal tolerance testing according to the plan displayed in Figure 1. A total of two hundred and forty fish, sixty from each temperature class, were needed for the complete test series. To aid in identifying the temperature class of a fish all fish were given an identifying mark. The fish were first anesthetized with methyl pentynol and then marked by the cold branding technique. The location of the brand indicated the temperature class of the fish, e.g., fish from class 1 (ambient river temperature) were branded on the right side, between the head and dorsal fin, above the lateral line; class 2 (river temperature plus 1.7° C) right side, below dorsal fin and above the lateral line; etc. Following the branding, one week was allowed the fish for recovery and to assure that the brand had become clearly visible. At the end of the week the fish were anesthetized again and carefully weighed. Then each temperature class was divided in half; each half composed of equal body weight distribution. One half of each temperature class was returned to the original holding tanks to await commencement of the thermal tolerance tests while the second half of each class was placed in a common acclimation tank. The water in the acclimation tank at the time the fish were introduced was slightly above river temperature and over a five day period was raised to 12° C. Once this temperature was reached the fish were held there for three weeks;

after which these fish were subjected to thermal tolerance tests. In the remainder of this paper the fish used in the first set of tests are referred to as Group I, while those fish acclimated to 12° C prior to the tests are referred to as Group II.

The thermal tolerance tests were conducted in a fiberglass trough measuring 4' x 15" x 15"; filled to a depth of 12". To obtain the desired water temperature for the various tolerance tests, heated and chilled water were supplied at constant pressure to a mixing valve. This valve allowed the investigator to accurately select the water temperature required. Once set, the mixing valve would hold that temperature for several hours without requiring adjustment. Normally the test temperature fluctuations were held within $\pm 0.1^{\circ}$ C. Before the water entered the test trough it was passed over a bubble tray which thoroughly frothed the tempered water and assured that it was air equilibrated. The flow rate of the water through the test trough was set at 8 gallons per minute. Thus a total exchange of the water in the trough occurred every 4.7 minutes. To eliminate thermal stratification the water in the trough was thoroughly mixed.

The thermal tolerance of the fish from Groups I and II were tested in the same manner, i.e., by recording the time until loss of equilibrium and the time until death at three lethally high water temperatures. The lethal temperatures used were 25, 27, and 29° C. Loss of equilibrium is here defined as that point at which the fish could not coordinate its movements sufficiently to remain upright. The time of death was defined as the cessation of observable movement of the operculum, mouth, or fins. At this point each fish was gently prodded in the peduncle area and was

considered dead if there was no response. In all tests the events were timed on an electric elapsed time clock and read to the nearest tenth of a minute.

The tests were conducted in blocks, i.e., four fish, one from each temperature class were tested at one time. This was done to reduce irregularities between the testing of the four different temperature classes. As four fish were selected for a test block care was taken to choose fish of the same size. After the last fish in each block died the four fish were weighed and measured.

RESULTS

The temperature of the incoming water to the laboratory for the month previous to the commencement of the tests averaged 4.0° C with a minimum temperature of 2.7° C and a maximum of 6.0° C. These temperatures were recorded between mid-December and mid-January; a time of the year when the Columbia River temperatures are nearing their seasonal minimum.

The mean postmortum weights of all the fish testes^d in both Groups I and II appear in Table 1, along with the standard error of the means. The weights are stated in grams and each mean represents 10 fish. The total weight range for Group I was 48 grams to 612 grams. Group II fish ranged from 61 grams to 796 grams. Analysis of variance was performed for the twelve combinations of test temperatures and rearing temperatures in both Groups I and II and no significant differences were found. The mean weight of all fish in Group I was 300 ± 44 grams and the Group II mean was 319 ± 49 grams. No significant difference was demonstrable here.

The geometric mean times to loss of equilibrium and the 95% confidence limits are presented in Table 2. The table shows the results for all tolerance tests performed. To test for significant differences between the thermal resistance of the four rearing temperature classes the Duncan Multiple Range Test was applied. In the table the result of this test appears as a letter for each test combination. Of the four means corresponding to one test temperature within a group, those means with common letters are not significantly different. In only one series of tests, those at 25° C was there a significant difference within the series. Those fish reared at river temperature plus 2.6° C showed significantly longer times to loss of equilibrium. The data seen in Table 2 are presented in graphical form in Figure 2. The general lack of significant differences is more readily apparent when graphed out in this manner; as is the one significant point. While no significant differences were found before acclimation, the figure does reveal that the range in thermal resistance was considerably narrowed by thermal acclimation.

Table 3 contains the data for the geometric mean times to death for all fish in the experiment. Each mean represents ten fish. As before, the Duncan Multiple Range test was applied. In Group I there are a series of significant differences. At test temperatures of 25 and 29° C those fish reared at river temperature plus 2.2 and 2.6° C survived longer than those maintained at river temperature and at river temperature plus 1.7° C. Basically the same result was found at the lethal temperature 27° C but the differences were not quite so obvious. There appears to be a graduated series of death times.

Group II geometric mean death times show no significant differences. Within each test temperature series each rearing group falls within the same Duncan category.

The data presented in Table 3 has been treated graphically in Figure 3. Again, the result of 12° C acclimation is clear. The differences in thermal tolerance due to rearing temperature are removed.

An attempt was made to determine the relationship of fish weight to the time to thermal death and the relationship of time to loss of equilibrium and to thermal death. The calculated correlation coefficients appear in Table 4. Inspection of the table reveals that the only significant correlation between fish weight and time to thermal death is found at the high test temperature, 29.0° C. This relationship is found in all four temperature classes in both Groups I and II. In regard to the relationship of time to loss of equilibrium and time to death the results are less clear. No significant correlation was found at the highest test temperature, 25° C. A very strong positive correlation was found at this temperature in Group II.

DISCUSSION

One of the characteristics of fish growth is the striking disparity in the size of individuals of the same age. As the fish increase in age the variance in the size frequency distribution increases (Brown, 1946, 1951). The two year old steelhead used in this study demonstrated this size depensation. Their body size ranged from 48 to 612 grams in Group I and from 61 to 796 grams in Group II. A large size range presents a problem to the investigator when dealing with physiological processes which are weight related. Fry et al (1946) indicated a relationship between fish size and their sensitivity to thermal stress. It was

important therefore that in this investigation care be taken to select fish for test blocks according to their size. If this had not been the case possible biasing of the results may have occurred. The results presented in Table 1 show that for each combination of rearing temperature and test temperature, for both Groups I and II these efforts were successful.

The geometric mean times to loss of equilibrium for Group I fish, i.e., fish tested directly from the four rearing temperature classes, were shown in Table 2. No significant differences were demonstrable in these means and thus it would appear that the thermal histories played no role in this portion of the thermal tolerance of the steelhead. Inspection of Figure 2, however, reveals there was a change that occurred in the geometric mean times following acclimation to 12° C. It is the opinion of the author that the explanation for the lack of significant differences in the Groups I means is the result of the unclear nature of the actual point of loss of equilibrium and the variability in the individual response of the fish. These two factors introduce variability into the data and makes any demonstration of differences difficult at these slight differences in thermal history.

The time of equilibrium loss was noted when the fish become inverted and was powerless to right itself. The time of "roll-over" actually occurs shortly after the actual point of equilibrium loss. Since equilibrium loss is not an instantaneous occurrence and can only be noted after the fact a more precise measure of equilibrium loss is needed if it is to be used in thermal tolerance tests.

The effect of thermal history did become apparent in the thermal death times, as seen in Table 3 and Figure 3. Although the fish had

been experiencing the normal temperature fluctuations that occur in natural waters the average temperatures for the four thermal classes remained on the thermal regime described above. The day to day fluctuations of water temperature are very slight and offer the fish no challenge; early data of Brett (1944) indicates that fish can make acclimatory changes at the rate of 1° C per day. Although the differences in thermal history were slight, as they might be for fish from different hatcheries, the fish demonstrated differences in their abilities to cope with thermal stress. Acclimation of the fish to a common temperature of 12° C for three weeks removed the differences.

The results of this study indicate that hatchery reared stocks of steelhead cultured under slight thermal differences respond to thermal stresses differently. According to the data presented here an average temperature difference of 2.2° C in the thermal history is required. If steelhead originating from two hypothetical hatcheries, whose water supplies differed in average temperature by this amount, were released at the same site in the Columbia and subsequently faced a thermal challenge, those from the warmer history would be significantly more resistant. After the two stocks of fish had been in the river for a period of three weeks the differences due to thermal history would have been eliminated.

Significant correlations were found between fish weight and time to thermal death in both Groups I and II at a test temperature of 29° C. The correlation coefficients were all positive; one was significant at the 0.05 level and the remaining seven were significant at the 0.01 level. These results are interpreted to mean that the larger fish are able to survive longer at the higher lethal temperatures. The explanation for this is that the large fish derives protection against the large ΔT° C

by virtue of its mass. When the fish is placed in the test chamber its body is essentially at the temperature of the holding tank from which it came. After it enters the test chamber there is a time lag while the body mass equilibrates with the test temperature. The large fish take sufficiently longer to reach equilibrium and thereby are somewhat protected from the acute lethal thermal effects as a result of their mass. At the lower lethal temperatures the correlation is not evident.

The correlation coefficients calculated for the relationship between time to loss of equilibrium and time to thermal death revealed an apparent tendency for significant positive relationship at the lower lethal temperatures. There is considerable variability in the mean time to death at the various lethal temperatures used and, as was pointed out earlier, this is also true of the loss of equilibrium times. At the high test temperatures the two events happen in very rapid succession and the scatter in the data makes it difficult to find any relationship. As the time scale of the events leading to thermal death is expanded by using lower test temperatures the relationship between loss of equilibrium time and thermal death time becomes clear.

SUMMARY

Steelhead trout eggs were hatched and the fish raised for two years according to the temperature regime: ambient Columbia River temperature, ambient river temperature plus 1.7, 2.2, and 2.6° C. At the end of the two year period the four temperature classes were tested for thermal resistance to three lethal temperatures; 25.0, 27.0, and 29.0° C. Significant differences were found between temperature classes in the time to thermal death but not in the time to loss of equilibrium.

Acclimation of a portion of each temperature class to a common 12° C removed the differences in thermal death times.

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BIBLIOGRAPHY

- Ayerst, J. Personal communication. Washington State Department of Game, 1971.
- Brett, J. R. Temperature tolerance in young Pacific salmon, genus Oncorhynchus. J. Fish. Res. Bd. Canada, 9:265-323, 1952.
- Brett, J. R. Some lethal temperature relations of Algonquin Park fishes. Univ. of Toronto Studies #52. Ontario Fish. Res. Lab. Pub. #63, 49 pp., 1944.
- Brown, M. E. The growth of brown trout (Salmo trutta, Linn.) I. Factors influencing the growth of trout fry. J. Exptl. Biol. 22:118-129, 1946.
- Brown, M. E. The growth of brown trout (Salmo trutta, Linn.) IV. The effect of food and temperature on the survival and growth of fry. J. Exptl. Biol. 28:473-491, 1951.
- Ebel, W. J. Supersaturation of nitrogen in the Columbia River and its effects on salmon and steelhead trout. U.S. Dept. of Int. Fish. Bull. Vol. 68(1):1-17, 1969.
- Fry, F. E. J., Hart, J. S., and Walker, K. F. Lethal temperature relations for a sample of young speckled trout, Salvelinus fontinalis. Univ. of Toronto Studies Biol. Ser. #54. Fish. Res. Lab. Pub. #66, 35 pp., 1946.
- Hart, J. S. Lethal temperature relation of certain fish of the Toronto region. Trans. Roy. Soc. Can. 41:57-71, 1947.
- Morris, R. W. Body size and temperature sensitivity in the cichlid fish, Aequidens portalegrensis. Am. Nat. 96:35-50, 1962.

Table 1. Mean weights of Adult Steelhead trout acute thermal resistance groups.
 (10 fish per test, weights in grams \pm standard error of the mean)

Group	Rearing temp. °C	Test Temperatures		
		25° C	27° C	29° C
I	River temp.	256 \pm 24	315 \pm 48	305 \pm 49
I	RT + 1.7	281 \pm 37	337 \pm 59	308 \pm 54
I	2.2	290 \pm 31	319 \pm 41	308 \pm 47
I	2.6	268 \pm 37	309 \pm 51	301 \pm 40
II	River temp.	330 \pm 52	379 \pm 67	259 \pm 36
II	RT + 1.7	334 \pm 32	349 \pm 51	274 \pm 38
II	2.2	350 \pm 52	319 \pm 49	269 \pm 42
II	2.6	343 \pm 65	327 \pm 43	286 \pm 45

Overall Means Group I 300 \pm 44, Group II 319 \pm 49.
 Analysis of variance revealed no significant differences
 12 tests in Group I or II.

Table 2. Geometric mean loss of equilibrium times for steelhead trout acute thermal resistance tests. (10 fish per test, times in minutes, with 95% confidence limits) Group I reared at temperatures indicated, Group II after 12° C acclimation.

Group	Rearing Temp, °C	Test Temperatures								
		25° C			27° C			29° C		
I	River temp.	13.3	(9.3-19.1)	A*	4.6	(3.2-6.5)	A	3.1	(2.4-3.9)	A
I	RT + 1.7° C	21.0	(10.3-42.7)	A	5.1	(4.0-6.6)	A	2.9	(2.4-3.6)	A
I	2.2	23.0	(10.3-51.3)	A	4.9	(3.6-6.6)	A	3.3	(2.9-3.8)	A
I	2.6	42.9	(19.8-92.8)	B	5.7	(4.5-7.3)	A	3.8	(3.4-4.3)	A
II	River temp.	411	(223-756)	A	19	(15-26)	A	4.3	(3.5-5.2)	A
II	RT + 1.7° C	447	(246-814)	A	17	(13-22)	A	4.5	(3.7-5.4)	A
II	2.2	435	(225-843)	A	17	(11-27)	A	4.3	(3.6-5.2)	A
II	2.6	495	(276-888)	A	19	(14-26)	A	4.5	(4.0-5.1)	A

* Duncan multiple range test. Within a group of four means those with a common letter are not significantly different (0.05 level).

Table 3. Geometric mean death times for adult steelhead trout acute thermal resistance test 5. (10 fish per test, times in minutes with 95% confidence limits) Group I reared at temperatures indicated, Group II after 12° C acclimation.

		Test Temperatures								
Group	Rearing Temp, °C	25° C			27° C			29° C		
I	River temp.	46.9	(32.1-68.6)	A*	12.0	(8.9-16.1)	A	5.6	(4.5-7.0)	A
	RT + 1.7	54.2	(33.7-87.1)	A	14.3	(11.6-17.5)	AB	6.0	(5.1-7.1)	A
	2.2	94.2	(74.3-119.5)	B	17.1	(14.3-20.5)	BC	6.8	(5.7-8.0)	B
	2.6	101.1	(66.8-152.9)	B	20.2	(18.5-22.0)	C	7.1	(5.9-8.6)	B
II	River temp.	416	(244-709)	A	38	(31-46)	A	6.8	(5.2-8.9)	A
	RT + 1.7	449	(268-753)	A	30	(26-34)	A	7.9	(6.4-9.8)	A
	2.2	455	(257-805)	A	32	(25-42)	A	7.2	(5.9-8.8)	AB
	2.6	472	(299-747)	A	35	(26-46)	A	8.9	(7.3-10.9)	AB

Duncan multiple range test. Within a group of four means those with a common letter are not significantly different (0.05 level).

Table 4. Correlation coefficients of weight-time to death and time to death-equilibrium loss for steelhead trout acute thermal resistance tests. (10 fish per test)

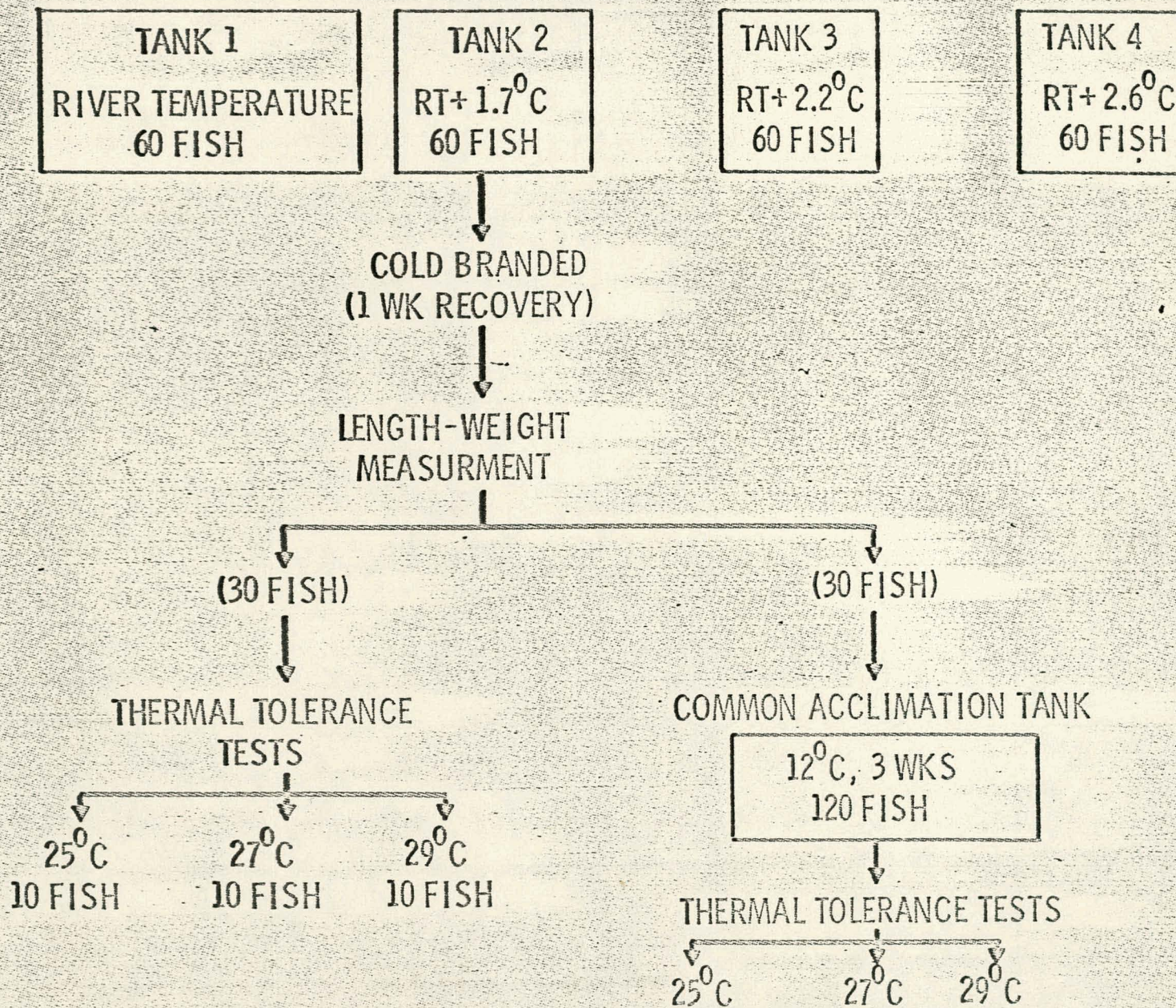
Group	Rearing Temp., °C	Weight-death time			Death-equilibrium loss times		
		25° C	27° C	29° C	25° C	27° C	29° C
I	River temp.	.18	.61	.97**	.10	.93**	.37
I	RT + 1.7	-.21	-.16	.95**	.85**	.61	.10
I	2.2	-.41	-.17	.85**	.16	.37	.42
I	2.6	-.43	-.11	.77**	.76**	-.09	.60
II	River temp.	.21	-.34	.70*	1.00**	.48	.34
II	RT + 1.7	-.23	-.41	.77**	1.00**	.59	.02
II	2.2	-.20	-.04	.75**	1.00**	.95**	.22
II	2.6	.15	-.28	.68**	1.00**	.59	.23

* Correlation significant at .05 level

** Correlation significant at .01 level

FIGURE 1.

STEELHEAD TROUT THERMAL TOLERANCE EXPERIMENT FLOW CHART



GEOMETRIC MEAN TIME TO LOSS OF EQUILIBRIUM ADULT STEELHEAD

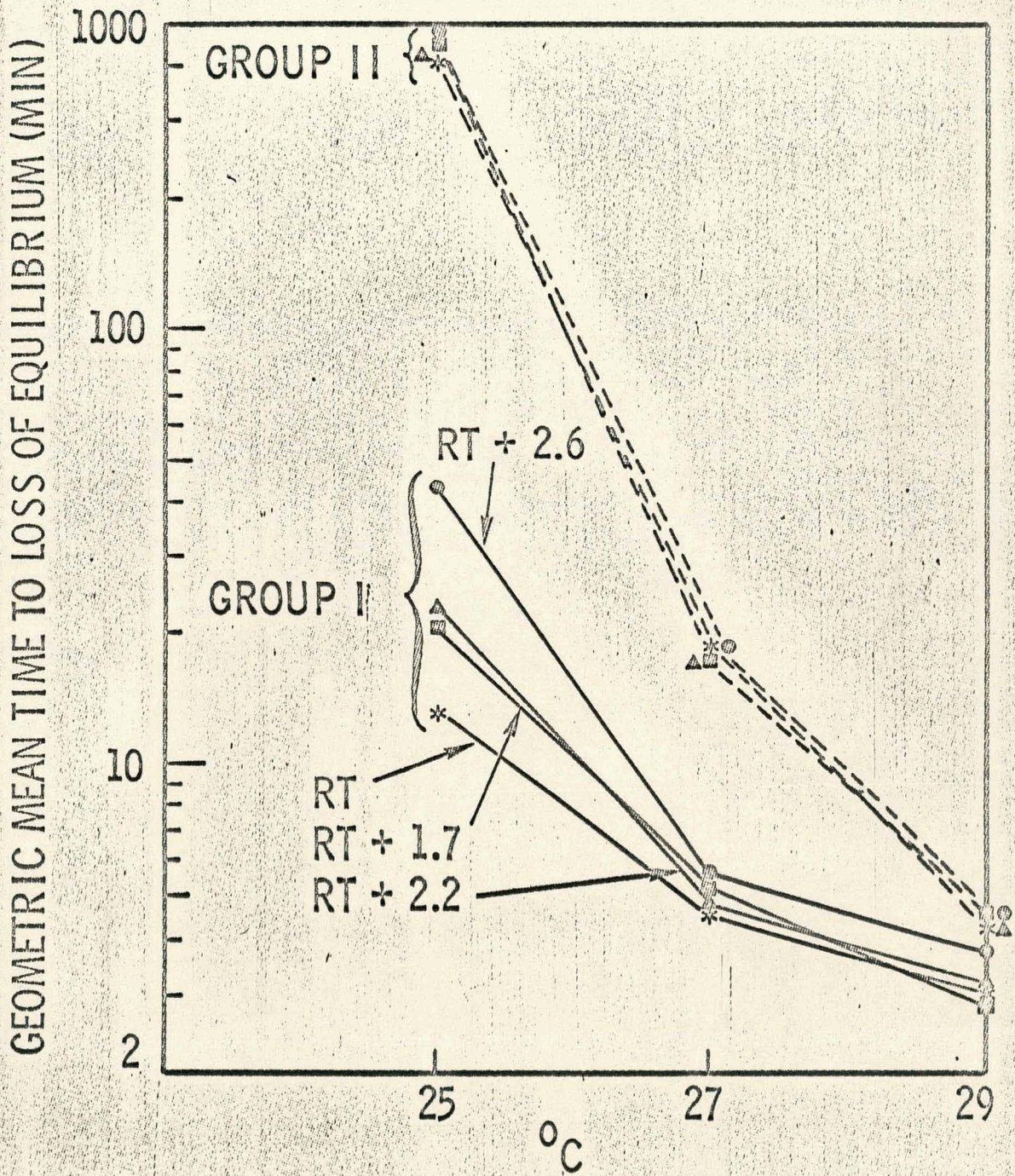


FIGURE 2.

GEOMETRIC TIMES TO DEATH ADULT STEELHEAD

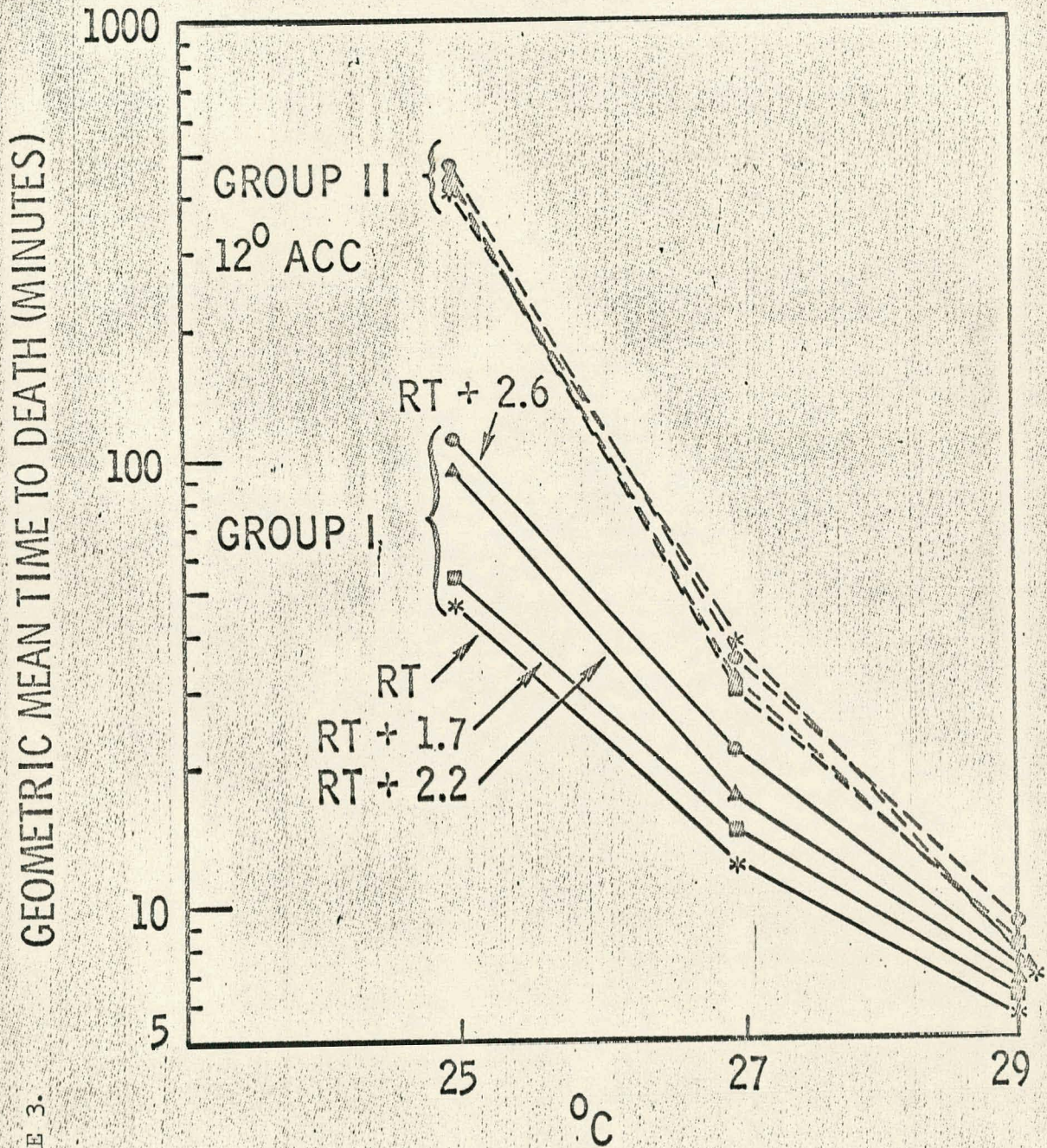


FIGURE 3.