Battelle

Pacific Northwest Laboratories
Richland, Washington 99352

AEC Research and Development Report

RELATIONSHIPS BETWEEN EQUILIBRIUM
LOSS AND DEATH AS RESPONSES OF
JUVENILE CHINOOK SALMON AND RAIN-
BOW TROUT TO ACUTE THERMAL SHOCK

NOVEMBER 1972

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately-owned rights.

PACIFIC NORTHWEST LABORATORY
operated by
BATTELLE
for the
U.S. ATOMIC ENERGY COMMISSION
Under Contract AT(45-1)-1830

Printed in the United States of America
Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, Virginia 22151
Price: Printed Copy \$3.00; Microfiche \$0.95

RELATIONSHIPS BETWEEN EQUILIBRIUM LOSS
AND DEATH AS RESPONSES OF JUVENILE CHINOOK SALMON
AND RAINBOW TROUT TO ACUTE THERMAL SHOCK*

Charles C. Coutant**

and

John Mark Deant†

Ecosystems and Biology Departments

November 1972

* Work performed under United States Atomic Energy Commission
Contract AT(45-1)-1830 (Environmental Sciences Branch, Division
of Biology and Medicine) with Battelle.

** Present Address: Ecological Sciences Division, Oak Ridge National
Laboratory, Oak Ridge, Tennessee 37830

† Present Address: Baruch Institute, University of South Carolina,
Columbia, South Carolina 29208

BATTELLE
PACIFIC NORTHWEST LABORATORIES
RICHLAND, WASHINGTON 99352

RELATIONSHIPS BETWEEN EQUILIBRIUM LOSS
AND DEATH AS RESPONSES OF JUVENILE CHINOOK SALMON
AND RAINBOW TROUT TO ACUTE THERMAL SHOCK

Charles C. Coutant
and
John Mark Dean

ABSTRACT

Loss of equilibrium by fishes is discussed as a manifestation of heat shock that is critical to survival of fishes. While similar to another endpoint of thermal resistance, the critical thermal maximum (CTM), the Equilibrium Loss Dose (ELD) defines the two pertinent variables, temperature and duration of exposure. In juvenile rainbow trout and Chinook salmon, geometric mean ELD and death dose were statistically distinct at high lethal temperatures, where ELD was 30 to 60 percent of the death dose. At low lethal temperatures, ELD was approximately 90 percent of the death dose, and the two endpoints were not statistically distinct. A cubic model for the ratio of ELD to death dose is presented.

CONTENTS

ABSTRACT	ii
INTRODUCTION	1
MATERIALS AND METHODS	4
RESULTS	5
Chinook Salmon	5
Rainbow Trout	7
DISCUSSION	8
ACKNOWLEDGMENTS	10
REFERENCES CITED	11
APPENDIX A: TABLES AND FIGURES	A.1

RELATIONSHIPS BETWEEN EQUILIBRIUM LOSS
AND DEATH AS RESPONSES OF JUVENILE CHINOOK SALMON
AND RAINBOW TROUT TO ACUTE THERMAL SHOCK

Charles C. Coutant and John Mark Dean

INTRODUCTION

Heat death of poikilotherms has been observed to follow a common pattern which includes, in sequence, loss of equilibrium, coma, and physiological death. While these observations have been made on several species of fishes, amphibians and reptiles, variations in experimental procedures and data analyses have led to contradictory conclusions and confusion in resulting principles. We believe these principles are important to understanding the thermal relations of aquatic populations, but find their presentation confused in the scientific literature now available. One unfortunate result of this situation is the difficulty in useful application of research on heat death to thermal pollution control programs. It is the purpose of this paper to 1) clarify certain concepts of equilibrium loss, which have evolved from research on thermal death, and 2) present data on the temporal relationship between equilibrium loss and death of Chinook salmon and rainbow trout.

Heat death has been studied more extensively in fishes than in other taxa, but only a few authors have attributed much significance to the behavioral events preceding death. Huntsman (1942) describes equilibrium losses in Atlantic salmon migrating into warm ($>30^{\circ}\text{C}$) streams in Nova Scotia. Here, loss of equilibrium was soon followed by cessation of swimming and death, both in the field and in laboratory tests with small salmon. Doudoroff (1942), reporting on laboratory experiments with *Girella nigricans* at constant lethal temperatures, noted an initial stimulation, then loss of equilibrium, and finally a permanent cessation of swimming and respiration which he termed "heat coma." He also noted differences in the timing of equilibrium loss with temperatures that were rapidly and slowly lethal. Brett (1944), also studying thermal resistance

at constant lethal temperatures, noted loss of equilibrium control and a tendency to roll up on one side in six species of fish, including five Cyprinidae and one Ictaluridae from Ontario. He also described the symptoms referred to by Doudoroff as coma. Further field observations of equilibrium losses under thermal stress were reported by Bailey (1955) and Threinen (1958). Tsukuda and Ohsawa (1958) reported both "spasmodic activity" and heat coma as events preceding death of the guppy (*Lebistes reticulatus*) when temperatures were raised slowly to the lethal point. Onset of heat coma was used by these authors as a convenient endpoint in thermal resistance studies. Hoff and Westman (1966) report "disturbances in equilibrium" as a principal feature of heat death at constant lethal temperatures in three species of marine fishes, the common silverside (*Menidia menidia*), the winter flounder (*Pseudopleuronectes americanus*), and the northern swellfish (*Spheroides maculatus*).

Working with desert reptiles independently of fisheries researchers, Cowles and Bogert (1944) introduced the concept of a "critical thermal maximum" and a "critical thermal minimum" to describe, for high and low limits of a species' tolerance, "the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death." The point was reached as the result of slow heating (or cooling) to the temperature of equilibrium loss. Lowe and Vance (1955), in further studies of reptile heat tolerances, have equated the critical thermal maximum (CTM) with the upper incipient lethal temperature of fishery researchers (e.g., Fry, Hart and Walker, 1946), stating that from the "ecological and evolutionary point of view" the CTM is the lethal point. Hutchison (1961), however, points out in his research with salamanders that despite the probable ecological significance of the CTM, it cannot be equated with the ultimate incipient lethal temperature since the behavioral response (CTM) must precede physiological death. He also recognizes that the CTM is experimentally derived in a very different manner, being a combined result of two variables, time and temperature, yet providing only

temperature as an endpoint. Recently, the CTM has been applied as a criterion of thermal tolerance in fishes by Lowe and Heath (1967) for the desert pupfish, *Cyprinodon macularius*, and by Heath (1967) for several species of inshore species in the northern Gulf of California.

A concept of a critical exposure to heat, which causes equilibrium loss, would seem to be of paramount significance in understanding the thermal relations of aquatic populations. It is increasingly recognized that the demise of animal populations is not absolutely dependent upon the physiological death limits of individuals, but upon broad ecological considerations such as breeding densities and predator-prey relationships. Equilibrium loss in the natural environment is a critical occurrence for an organism, for it significantly reduces the ability to escape from the thermal conditions which are causing distress and imminent death (as suggested by Cowles and Bogert) and it provides important stimulatory cues to predators. The latter effect may be a particularly important feature in fishes. Mossman (1955) cites several points of evidence that suggest release of predator attack by any behavior associated with weakness. Spasmodic swimming activity may propel the individual from the localized zone of initial thermal effects (warm shallows, heated industrial discharges, warm tributaries, etc.) and allow recovery, but the fish is highly subject to predation throughout this movement.

The CTM, however, is inadequate as a quantitative description of equilibrium loss because, as Hutchison (1961) discussed, its determination involves two variables, time and temperature, that are not described by the resulting temperature maximum. We suggest that an "equilibrium loss dose" (ELD) is a more applicable description, derived from resistance time experiments of Fry, Hart and Walker (1946) and Brett (1952). In these determinations, temperature and time to loss of equilibrium are specified and the use of the word "dose" implies a time-dependent function. Both the experimental method and the system of reporting such doses are well established in fisheries literature and have their basis in the statistical analysis of pharmacology (Bliss 1937). The predictive utility of such

quantitative data was discussed by Fry et al. (1946), Harty et al. (1967), and Jaske, Templeton and Coutant (1970).

In view of the preliminary discussion of the importance of ELD, we now present data on the relationships between ELD and doses to physiological death at constant lethal temperatures. At a given constant lethal temperature, we may simply compare the times of equilibrium loss and death, for doses will be proportional.

Two somewhat divergent approaches may be taken to the problem; namely, that of the physiologist interested in the individual responses, and that of the ecologist who wishes to estimate the response of a population on the basis of tests with selected individuals. One may argue that they may be statistically equivalent, but the viewpoint of the investigator will influence his judgment of the utility of the results. We are concerned primarily with population effects and ecological principles in this paper, but we shall include discussion of individual variability as well.

MATERIALS AND METHODS

Juvenile Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), and rainbow trout, *Salmo gairdneri* (Richardson), were used in these experiments. Juvenile Chinook salmon from the fall run into the Columbia River were raised in our hatchery from parents obtained from the Washington Department of Fisheries in the spawning channel at Priest Rapids Dam (638.9 km from the river mouth). Incubation and rearing were conducted at seasonal day lengths (building illumination was controlled by a photoelectric switch) and at temperatures close to that of the river (the hatchery was supplied by pumping directly from the Columbia). Rainbow trout were similarly raised in our hatchery from a brood stock originating several generations previously in the hatchery at Naches, Washington. Juvenile Chinook and rainbow were acclimated to 15 °C in running water in the laboratory for a minimum of three weeks prior to testing.

Geometric mean times to loss of equilibrium and death at selected lethal temperatures were determined by an experimental method similar to

that of Brett (1952) in running water in a specially designed test apparatus. The endpoints for equilibrium loss and death were, respectively, complete body inversion and cessation of opecular beating. Generally, lots of ten fish were used for each test temperature. Most tests are replicated. The results reported herein are derived from equilibrium loss and death times for 1480 juvenile Chinook and 80 juvenile rainbow.

RESULTS

CHINOOK SALMON

Figure 1 presents cubic models of the geometric mean times to equilibrium loss and death in 360 juvenile Chinook with identical rearing history. The data did not fit the linear similog model of Brett (1952), but were more closely approximated by these cubic models. The jog for EL near 29.5 to 30 °C is an artifact of modelling. Progressive divergence of the two lines with increasing temperature is apparent. Confidence limits were not included for the curves due to paucity of data, but "t-tests" were conducted on the individual data points. These indicate that thermal doses to this group of similar fish sufficient to cause equilibrium loss and death are statistically different at the 95% confidence level at temperatures higher than about 27.7 °C. Between this temperature and 26.0 °C, the values are certainly different for each individual and each lot of fish, but the variabilities among lots in both geometric mean death and equilibrium loss times are sufficiently great that the confidence intervals of each overlap. Details of the cubic models are presented in Table 1.

Equilibrium loss and death data were also obtained for 1120 additional Chinook juveniles, derived from several groups with different thermal histories during incubation and rearing, but also acclimated to 15 °C. The cubic models for equilibrium loss and death times for all juvenile Chinook are illustrated in Figure 2. The pattern is similar to that for the single group in Figure 1, but it has a larger data base, and is, therefore, more complete. Confidence intervals (95%) are illustrated for averaged times at

each test temperature (vertical bars) and for the cubic models (hatched area). The two criteria for thermal effect can be considered statistically distinct (based upon the cubic models) at about 27.25 °C.

Figures 3 and 4 illustrate ratios of geometric mean equilibrium loss times to geometric mean death times. Figure 3 considers, first, the sample of Chinook salmon with similar rearing history. At lower lethal test temperatures, the equilibrium loss doses are high percentages (above 90%) of the doses causing death. The ratio drops markedly with increasingly higher test temperatures, to levels between 30 and 60%. The variability also increases, although there are too few points to obtain an adequate measure of it. Above 29 °C, the downward trend is apparently reversed (note 95% confidence intervals) and the percentage returns to somewhat higher levels.

The pattern which emerged for these Chinook was tested by including data obtained on the additional 1120 fish with differing thermal histories (Figure 4). As with thermal resistance times, the result was similar, but strengthened by many additional ratios. These results are approximated by the cubic model

$$\frac{EL}{D} = 1.05300 + 0.104565 (T-25) - 0.158224 (T-25)^2 + 0.0237243 (T-25)^3.$$

Sufficient points were available in the pooled Chinook data to measure relative variability among test temperatures. Pearson's coefficient of variation (Moroney, 1951), plotted in Figure 4B, illustrates high variability in the midrange of lethal temperatures. The variability at the extreme high and low lethal temperatures is very small for most biological data.

Another approach relating ELD to the death dose (DD) is to fit a regression line of ELD versus DD independent of test temperature. An interesting relationship appears with Chinook between 26.0 °C and 28.5 °C test temperatures for which there were many replications of tests at each temperature. The regression equation,

$$DD = 605 + 1.012 (ELD)$$

Residual Standard Deviation = 150

0.95 Confidence Limits on the Slope:

$$p [0.978 < \beta < 1.046] = 0.95$$

suggests that in this temperature range, fish die about 600 sec (10 min) after they lose equilibrium regardless of the specific test temperature. There may be a physiological basis for this apparent constancy. The few tests with rainbow trout do not show this relationship.

RAINBOW TROUT

Although fewer data are available for this species, there appears to be a pattern similar to that for Chinook. Times to equilibrium loss and death of a small sample of siblings of this species are plotted in Figure 5, along with 95% confidence intervals of the points. As with young Chinook, the two parameters appear distinct at the 95% confidence level at higher lethal temperatures only. In this species, the break occurs slightly above 29 °C. That this level is higher than the similar level for Chinook is consistent with the generally increased thermal tolerance of rainbow trout as compared to that of Chinook.

The ratios of equilibrium loss times to death times for these fish are shown in Figure 4. Lower lethal temperatures produced large ratios which declined progressively with increasing temperature. There is no evidence of a return to higher ratios, as seen in Chinook, but the temperature for this effect may not have been reached. Displacement of the ratios towards higher temperatures is again consistent with the higher thermal tolerance of this species compared to Chinook.

All of the above comparisons have been made on essentially a "population" basis, even though the population size for which geometric mean values were derived was generally ten fish. When analyzed on an individual basis, the results offer little correlation between ELD and DD, even at high test temperatures. Table 2 presents the results of correlation analyses between times to equilibrium loss and death for

individual juvenile Chinook from four rearing lots, each tested at three temperatures. Among 12 comparisons, only two indicate significant correlations (at the 95% confidence level). These results suggest that individual variation in relative timing of ELD and DD is large, but that populations may still respond predictably.

DISCUSSION

The theoretical importance of equilibrium loss doses to populations of aquatic organisms should be apparent from the introduction. Perhaps the first question to arise from the results presented is that of the pertinence of distinguishing between these two responses, ELD and death, when they are so close, especially at low lethal temperatures.

The endpoint for equilibrium loss chosen for these comparisons (i.e., body inversion) was selected for its relatively high precision of identification and for its approximation of the heat coma condition observed by other authors. We recognize that during exposure of fish to a lethal temperature, physical coordination is lost somewhat progressively until fish completely lose control and invert. The ecological significance of equilibrium loss in the environment may be manifested at doses significantly less than those recorded in this study. This appears to be the case in more recent experiments with predators (Coutant, unpublished). To answer the question posed for low lethal temperatures, it would appear that, with the criterion chosen in this study, the equilibrium loss dose does not differ to any meaningful extent from the death dose reported by many other authors. We suspect this to be more a criticism of our selection of a proper criterion, rather than the reality of equilibrium loss effects in nature.

At higher lethal temperatures, differences between doses leading to equilibrium loss and death are greater (equilibrium loss time is only 30 to 60% of death time) and more significant, even with use of the inversion criterion. For these higher lethal temperatures, we therefore suggest that there is a demonstrable and, we believe, pertinent

difference between thermal resistance measured by equilibrium loss and by death. This difference, and its pertinence, would be increased by an inadequate selection of the endpoint, as suggested above.

The second possible question concerns the utility of another measure of thermal resistance when resistance time to death is well established in scientific literature. We believe that this utility is found in enhanced capability for quantitatively predicting the environmental effects of thermal additions to rivers, particularly from thermal plumes through which juvenile anadromous fish must pass. Quantitative predictions of such effects and their use in designing safe thermal discharges are discussed by Jaske, Templeton and Coutant (1970). We suggest that evaluations of thermal mixing zones on the basis of directly lethal doses alone are inappropriate. At a minimum, the equilibrium loss doses should be substituted, for it represents a critical thermal dose for survival of the organism (although it may still not be the critical dose in all situations). We do not, however, suggest that the quantitative utility of existing thermal resistance data is totally invalid. Rather, the tentative relationships between the two parameters, which we have derived, may be used as flexible "correction factors" for the presently abundant thermal death times.

ACKNOWLEDGMENTS

We thank Mr. Robert G. Genoway for conducting most of the experiments, Dr. John Thomas and Mr. Richard Olson for statistical analyses and modelling of the data and Mr. William L. Templeton for reviewing the paper.

REFERENCES CITED

- Bailey, R. M. 1955. Differential mortality from high temperature in a mixed population of fishes in southern Michigan. *Ecology* 36:526-528.
- Bliss, C. I. 1937. The calculation of the time-mortality curve. *Ann. Appl. Biol.* 24:815-852.
- Brett, J. R. 1944. Some lethal temperature relations of Algonquin Park fishes. *Publ. Ont. Fish. Res. Lab. No. 63, Univ. Toronto Stud., Biol. Ser., No. 52.* 1-49.
- Brett, J. R. 1952. Temperature tolerance in young Pacific salmon, genus Oncorhynchus. *J. Fish. Res. Bd. Canada* 9:265-323.
- Cowles, R. B. and C. M. Bogert. 1944. A preliminary study of the thermal requirements of desert reptiles. *Bull. Amer. Mus. Nat. Hist.* 83:265-296.
- Doudoroff, P. 1942. The resistance and acclimatization of marine fishes to temperature changes. I. Experiments with Girella nigricans (Ayres). *Biol. Bull.* 83:219-244.
- Fry, F. E. J., J. S. Hart and K. F. Walker. 1946. Lethal temperature relations for a sample of young speckled trout (Salvelinus fontinalis). *Univ. Tor. Stud., Biol. Ser., No. 54; Pub. Ont. Fish Res. Lab.* 66:5-35.
- Harty, H., et al. 1967. Nuclear Power Plant Siting in the Pacific Northwest. Final Report to the Bonneville Power Administration. Battelle-Northwest, Richland, Wash. 547 p.
- Heath, W. G. 1967. Ecological significance of temperature tolerance in Gulf of California shore fishes. *J. Arizona Acad. Sci.* 4:172-178.
- Hoff, J. G. and J. R. Westman. 1966. The temperature tolerances of three species of marine fishes. *J. Mar. Res.* 24:131-140.
- Huntsman, A. G. 1942. Death of salmon and trout with high temperature. *J. Fish. Res. Bd. Canada* 5:485-501.
- Hutchison, V. H. 1961. Critical thermal maxima in salamanders. *Physiological Zool.* 34:92-125.
- Jaske, R. T., W. L. Templeton and C. C. Coutant. 1970. Methods for evaluating effects of transient conditions in heavily loaded and extensively regulated streams. *Proc. 1969 ASME/AIChE Stream Pollution Abatement Conference, Rutgers Univ., 10-12 June 1969.*

- Lowe, C. H. and W. G. Heath. 1969. Behavior and physiological responses to temperature in the desert pupfish (Cyprinodon macularius). *Physiol. Zool.* 42:53-59.
- Lowe, C. H., Jr. and V. J. Vance. 1955. Acclimation of the critical thermal maximum of the reptile *Urosaurus ornatus*. *Science* 122:73-74.
- Moroney, M. J. 1951. Facts from Figures. Penguin Books, Baltimore, Md. 472 p.
- Mossman, A. S. 1955. Experimental studies of fitness as measured by vulnerability to predation. Ph.D. Dissertation, Univ. of Wisconsin, Madison, 134 p.
- Threinen, C. W. 1958. Cause of mortality of a midsummer plant of rainbow trout in a southern Wisconsin lake, with notes on acclimation and lethal temperatures. *Prog. Fish. Cult.* 20:27-32.
- Tsukuda, H. and W. Ohsawa. 1958. The heat and cold coma temperatures in Lebistes reticulatus as determined by raising and lowering water temperature at constant rates. *J. Inst. Polytech., Osaka City Univ., Ser. D.* 9:69-76.

APPENDIX A
TABLES AND FIGURES

TABLE 1. Relationships of Equilibrium Loss and Death of Juvenile Chinook Salmon to Shock Temperature

Model: $t = A + B(T-25) + C(T-25)^2 + D(T-25)^3$
 where
 t = geometric mean time
 T = test time

and

Curve	A $\pm$ S.D. ¹	B $\pm$ S.D.	C $\pm$ S.D.	D $\pm$ S.D.
Similar Chinook				
EL	23432.226 $\pm$ 1200	-15308.811 $\pm$ 950	3339.6027 $\pm$ 250	-165.90 $\pm$ 18
D	21512.979 $\pm$ 1125	-12726.496 $\pm$ 870	2516.809 $\pm$ 220	-242.76 $\pm$ 20
All Chinook				
EL	20663.092 $\pm$ 603	-13630.453 $\pm$ 415	3003.4067 $\pm$ 94.9	-220.39176 $\pm$ 7.2
D	20670.30 $\pm$ 413	-12607.78 $\pm$ 402	2586.12 $\pm$ 133	-177.6258 $\pm$ 1.5

¹S.D. = 1 Standard deviation

TABLE 2. Correlation Coefficients Between Equilibrium Loss and Death of Individual Juvenile Chinook Salmon in Four Rearing Lots of 10 Fish Each, Tested at Four Temperatures

Rearing lot	Test Temperature		
	<u>29.0</u>	<u>29.5</u>	<u>30.0</u>
2	.52	.51	.62
3	.31	.46	.90*
4	.48	.73*	.60
5	.21	.50 ¹	-.06

* Significant at .05 level

¹ Based on 9 fish

FIGURE 1. Cubic Models of Geometric Mean (GM) Times to Equilibrium Loss (EL) and Death (D) of Juvenile Chinook Salmon with Similar Rearing History (details in Table 1). Confidence limits (95%) of the points are shown.

FIGURE 2. Cubic Models of Geometric Mean Times to Equilibrium Loss (dashed) and Death (solid) of all Juvenile Chinook Salmon for Which Data were Available (details in Table 1). Confidence limits (95%) as shown for both points and lines.

FIGURE 3. Ratios of Geometric Mean Equilibrium Loss Times to Death Times for Juvenile Chinook Salmon with Similar Rearing History, and for Sibling Juvenile Rainbow Trout. Confidence Limits (95%) are shown for means, where applicable. Dashed lines were placed by inspection or through means.

FIGURE 4. A) Relationship Between Geometric Mean Equilibrium Loss and Death Times for all Juvenile Chinook Salmon for Which Data were Available. Means and 95% confidence intervals are shown for most test temperatures. The curve represents a cubic model for the data.

B) Coefficient of Variation for Ratios at Various Test Temperatures.

FIGURE 5. Geometric Mean Times (with 95% confidence limits) for Equilibrium Loss and Death of Juvenile Rainbow Trout

DISTRIBUTION

No. of
Copies

OFFSITE

- 1 AEC Chicago Patent Group
 G. H. Lee
- 194 AEC Technical Information
 Center
- 25 Oak Ridge National Laboratory
 C. C. Coutant (25)

ONSITE - HANFORD

- 1 AEC Chicago Patent Group
 R. W. Poteat
- 2 AEC Richland Operations Office
 W. E. Lotz
- 136 Battelle-Northwest
E. L. Alpen	D. E. Olesen
W. J. Bair	T. L. Page
C. D. Becker	R. S. Paul
G. M. Dalen	W. D. Richmond
M. J. Duever	M. J. Schneider
R. M. Emery	A. J. Scott
S. J. Farmer	C. L. Simpson
R. F. Foster	J. A. Strand
J. J. Fuquay	W. L. Templeton (100)
C. I. Gibson	T. O. Thatcher
W. A. Haney	R. Y. Ting
R. T. Jaske	B. E. Vaughan (2)
J. A. Lichatowich	Life Science Library (2)
J. M. Nielsen	Technical Information (5)
T. P. O'Farrell	Technical Publications (2)