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Title: THE EFFECTS OF FLUCTUATING TEMPERATURES ON THE LETHAL TOLERANCE OF COASTAL CUTTHROAT TROUT *(Salmo clarki clarki)*

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Abstract Approved: ______________________
Carl B. Schreck

The potential differences in the thermal tolerance of hatchery and wild coastal cutthroat trout *(Salmo clarki clarki)* acclimated to constant and fluctuating temperatures was investigated. Hatchery and wild cutthroat trout responded similarly to critical thermal maxima (CTM) tests and to constant and fluctuating lethal temperatures. Fish acclimated to 13-23°C diurnal temperature fluctuations had lower median survival times (MST) and CTM's than fish acclimated to 23°C. Fish acclimated to 13-25°C had MST's that were higher than those of fish acclimated to 23°C. Fish from both 23 and 13-23°C temperature regimes could withstand 13-27.0°C exposures for 1 wk. Two models used in predicting the mortality of cutthroat trout exposed to fluctuating lethal temperatures that utilize constant temperature bioassay information were found to be adequate for use with fish acclimated to a temperature regime that simulated a post-logging condition. Methods for selecting an equivalent constant level of acclimation for fish acclimated to a fluctuating temperature regime appear feasible.

The Effects of Fluctuating Temperatures on the Lethal Tolerance Limits of Coastal Cutthroat Trout *(Salmo clarki clarki)*

by

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INTRODUCTION

Seasonal and daily temperature variations in the aquatic environment which are outside the normal scope for growth, reproduction, and activity or the normal tolerance limits of fish (Fry 1971) may occur naturally or because of man's activities. Forest fires and drought can cause temporary or long-term increases in stream temperature. Increases in temperature can be attributed to point-sources such as steam-electric generating plants (Krenkel and Parker 1969) and non-point sources such as logging activities. Thermal pollution (Mathur 1976) from point and non-point sources often cause elevated mean temperatures as well as increases in the amplitude of daily temperature fluctuations (Krenkel and Parker 1969; Moring 1975).

My overall goal was to provide an understanding of the effects of elevated diel fluctuations in temperatures on the lethal tolerance limits of coastal cutthroat trout (*Salmo clarki clarki*). My objectives were to determine whether or not constant temperature bioassay information could be applied to two models to see if thermal death could be predicted for fish exposed to fluctuating lethal temperatures, and to determine whether or not a constant acclimation temperature equivalent could be found for fish acclimated to a fluctuating temperature regime. The model for these fluctuations was based on the thermal pollution that occurred under post-logging conditions on

Needle Branch Creek, a small coastal tributary stream (Moring 1975). In the actual case and in the laboratory simulated regime, low mean daily temperatures with slight diurnal fluctuations during the winter months were followed by an increase in both the daily mean and range of the diel fluctuation in temperature as summer approached. Since rate of acclimation is important in establishing a fish's temperature to tolerance, critical thermal maxima (CTM) were compared at various stages in the process of acclimation to the warmest constant and fluctuating temperature regimes. Similar comparisons were also made for acclimation to cooler water temperatures as might be encountered in the fall. Median survival times (MST) and incipient lethal levels (Warren 1971) were compared for fish acclimated to warm constant temperatures and warm temperatures with large diurnal fluctuations.

Data derived from bioassays conducted at constant temperatures were used to estimate lethal limits of fish exposed to fluctuating lethal temperatures. The estimates were compared to the observed lethal tolerance limits graphically. Potential differences in temperature resistance between hatchery and wild fish were also determined.

MATERIALS AND METHODS

Experimental Animals

Hatchery cutthroat trout eggs for the 1975 and 1976 experiments were transferred from the production supply at the Alsea Trout Hatchery, Alsea, Oregon, on 15 March 1975 and on 10 February 1976 respectively, to the Oregon Department of Fish and Wildlife Research Section Laboratory in Corvallis. The eggs were then disinfected with 1:150 Wescodyne[R] and water buffered with sodium bicarbonate and placed into Heath[R] incubators. Similar procedures were followed with wild cutthroat trout eggs obtained from Big Creek Salmon Hatchery, Astoria, Oregon. Hatchery and wild cutthroat trout eggs obtained in 1976 had similar thermal histories with 476 and 450 T.U.'s (temperature units represent the average daily temperature above freezing summed over the period of incubation) respectively upon arrival at the laboratory (Leitritz and Lewis 1976). All groups of eggs were incubated under a 7.5-10°C (nominal) fluctuating temperature regime to simulate natural stream conditions. Hatching took place on 25 March 1975 and 19 February 1976 for hatchery stocks and 17 March 1976 for wild stocks. Fish were subsequently fed Oregon Moist Pellet[R] and reared under the 7.5-10°C regime until they were used in experiments. Fish not used in experiments were maintained at the above temperature regime as controls. Mean length was 6.0 and 4.6 cm for hatchery and wild cutthroat trout, respectively, at the beginning of the experiments.

Experimental Apparatus

Five Honeywell® dual temperature controller-recorder units (model 604 P43DD-74-111-896) and 10 three-way mixing valves (Precision Products and Controls Research Model 6A-711-35M) provided water at desired temperatures to ten 140 L circular fiberglass aquaria, with the option of shunting water to three 357 L tanks that were used for acclimating large groups of fish to fluctuating temperatures. The temperature was controlled by pre-cut Plexiglas® cams. These were changed weekly to simulate seasonal and daily temperature changes with an accuracy of ±0.2°C of the desired temperature, even during large diurnally fluctuating temperature cycles. In addition to the tanks with programmable temperature controls, two other 357 L tanks were used for acclimating fish to constant temperatures. The 357 L tanks had an inflow of 6 L/min and the 140 L aquaria received 3 L/min. All tanks were fitted with stripping columns which maintained the percentage saturation of gas below 103% at all temperatures (Fig. 1).

Control fish were maintained in two 350 L tanks with an inflow of 12 L/min. The 7.5-10°C control regime was created by mixing 12°C well water and 7.5°C chilled well water using Dayton solenoid valves (Model 6X082) and a Dayton timer (Model 2E026). Temperature was recorded with either the Honeywell instruments, Partlow thermograph (Model RFHTT), or a Stolab (Model 911PL) electronic thermometer and Weston panel meter (Model 1230) to the nearest 0.1°C.

The water had a hardness of 84-99 mg/l (as $CaCO_3$), a pH

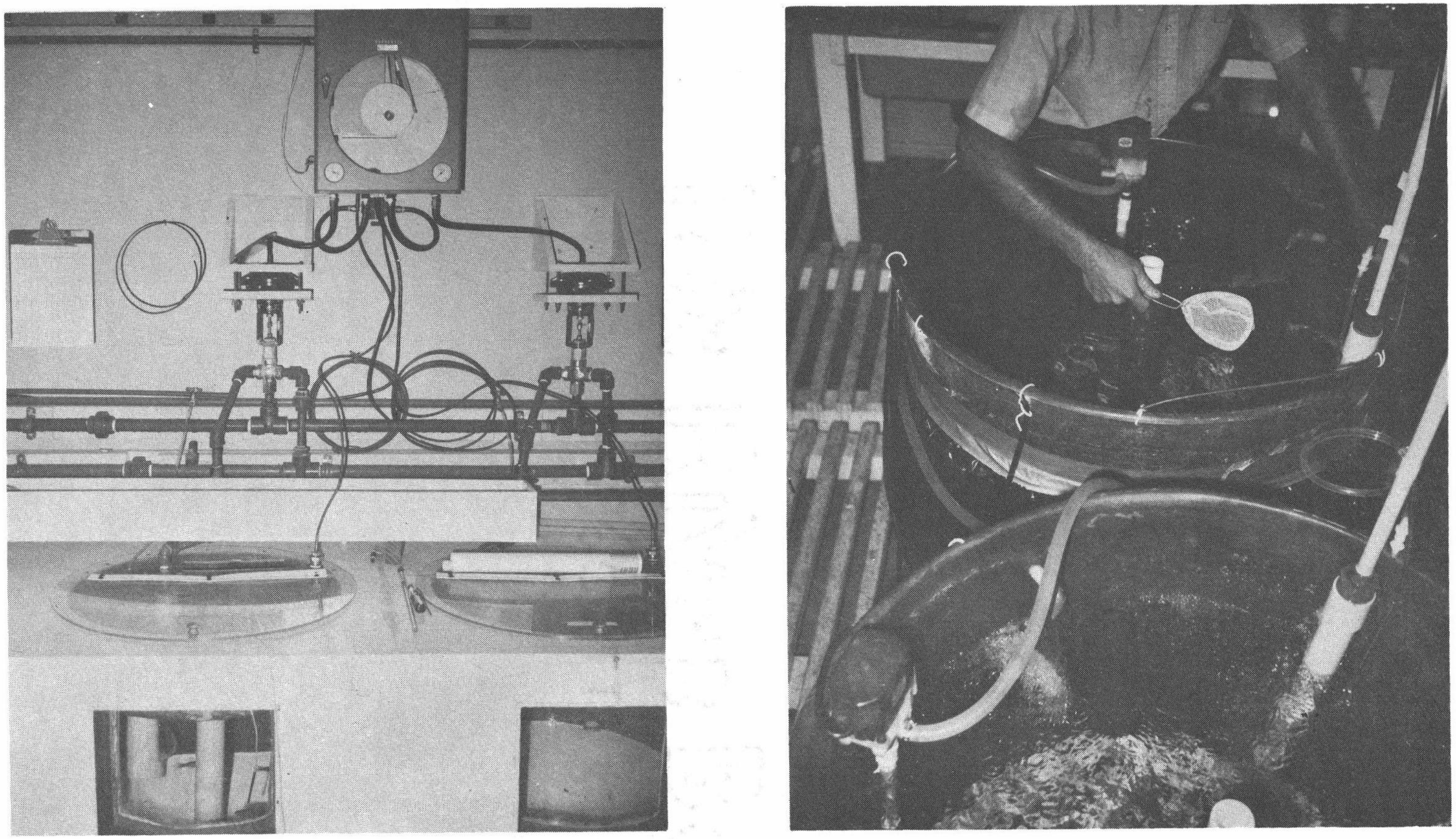

Figure 1. Experimental apparatus for providing controlled temperature environments.

(a) Controller-recorder and three-way mixing valve and two of the experimental tanks described in the text.

(b) Two of the 357 L tanks used in acclimating cutthroat trout to a temperature regime simulating a post-logging condition. Gas stripper columns are near the right side of the tanks.

of 7.2-7.5, and a dissolved oxygen concentration of 7.3-10.5 mg/L at 12°C (Lorz and McPherson 1976).

1975 Experiments

Constant temperature bioassays

On 28 May 1975, an experiment was initiated to determine if the response of Alsea Hatchery cutthroat trout to lethal temperatures was the same for fish acclimated to 23°C as it was for fish acclimated to 13-25°C. Four 357 L tanks with 1,000 fish each were used for acclimating two groups of fish to 23°C and two groups to 13-25°C temperature regimes. Beginning 28 May 1975, the fish were slowly acclimated 23 and 13-25°C over a period of 8 wks, after which constant temperature bioassays were conducted over a 10 wk period. Ten fish were used per bioassay and four replicates were made for each test temperature, which ranged from 26.0 to 28.0°C (nominal) in 0.5°C increments. The MST's were estimated using probit analysis. The regressions of MST against test temperature were compared using an analysis of covariance to determine if there was any effect due to acclimation.

1976 Experiments

Acclimation

Two basic acclimation schemes were employed for hatchery and wild cutthroat trout. On 21 and 22 May 1976, approximately 500 hatchery and 500 wild fish were placed in separate 357 L tanks at 10°C. Beginning 17 June 1976, the fish were slowly acclimated to a nominal temperature regime of 23°C in stages over a 7 wk period. After being held for 15 wk at 23°C, the fish were acclimated back to 10°C over a 7 wk period (Fig. 2a). Another test regime, also started on 17 June 1976, simulated exposure to known post-logging temperature conditions, and consisted of acclimating approximately 500 hatchery and 500 wild cutthroat trout in two additional 357 L tanks to 7.5-10°C; one stock per tank. Following the same time schedule as in the first acclimation scheme, the fish were slowly acclimated to a nominal temperature regime of 13-23°C using a daily increasing, dielly fluctuating temperature regime. After the fish had been held for the 15 wk period, they were acclimated to the 7.5-10°C regime over 7 wks (Fig. 2b). The diel cycles were sinusoidal in shape with maximum temperatures occurring between 1400 and 1800 h and minimum temperatures between 0200 and 0600 h PST.

Critical thermal maximum determination

The CTM was determined by taking five hatchery and five wild fish from each temperature regime and exposing them to a 0.125°C/min increase in temperature until the fish were observed to lose their equilibrium. Fish were considered to have lost equilibrium when they were no longer swimming upright. Tests were conducted between 1400 and 1800 h for fish acclimated to constant temperatures. Fish acclimated to fluctuating thermal regimes were tested from 1600 to 1800 h during the peak temperature of the diel cycle. These tests were conducted during the upward phase of acclimation of 23°C and 13-23°C regimes as well as during downward acclimation to 10 and

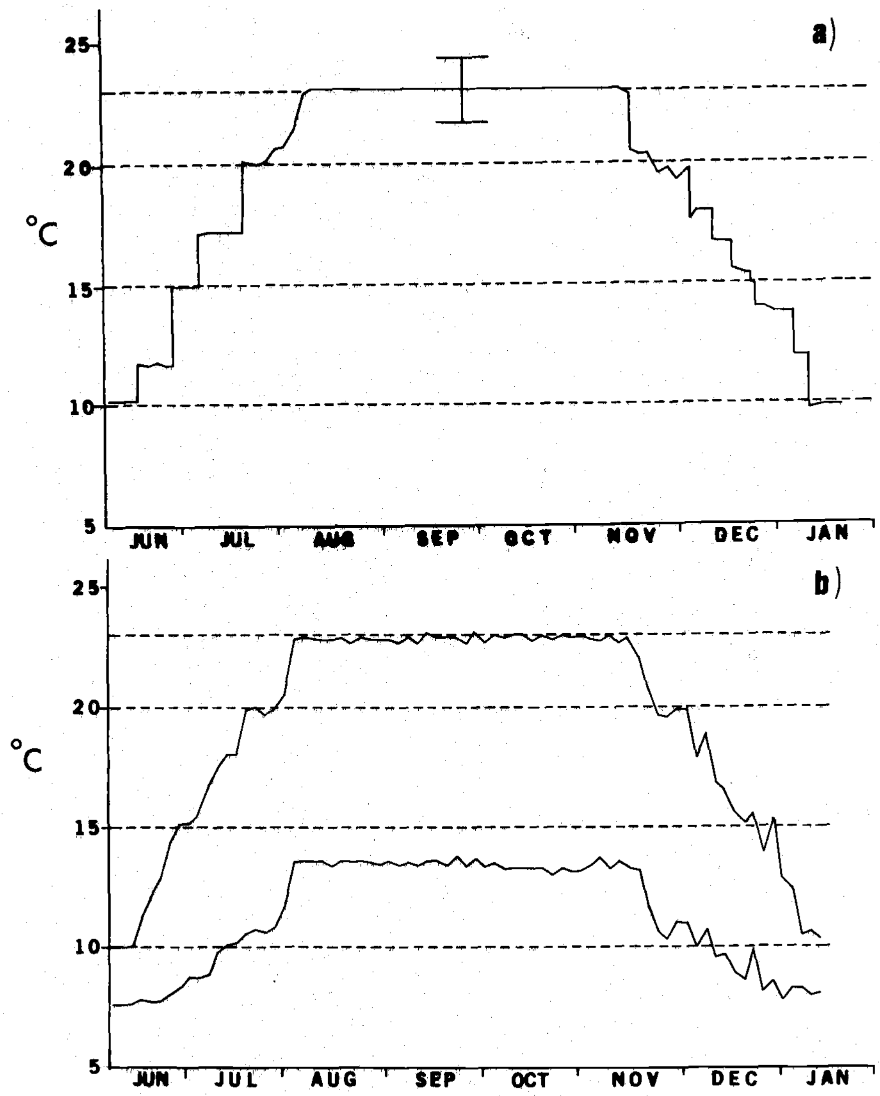

Figure 2. Basic acclimation regimes for wild and hatchery coastal cutthroat trout used in 1976 experiments. Dashed lines indicate levels where thermal maximum experiments were testes;

(a) Constant temperature acclimation regime. The vertical bars represent maximum and minimum range during summer temperatures.

(b) Gradually increasing (and decreasing) dielly fluctuating temperature regime simulating post-logging temperatures. Upper and lower solid lines represent maximum and minimum daily temperatures respectively.

7.5-10°C. Nominal acclimation temperatures at which the fish were tested were 10, 15, 20, and 23°C for fish acclimated in steps to constant temperatures; 7.5-10, 8.6-15, 10-20, and 13-23°C for fish acclimated to fluctuating temperatures. Each test was replicated once. In addition to the above determinations, hatchery, wild or both hatchery and wild fish from 7.5-10°C control tanks were tested periodically during the course of the experiment.

The rates of CTM change with time and mean CTM's for the above groups were compared using an analysis of covariance of the stock x acclimation x replication regressions (Neter and Wasserman 1974). Similar comparisons were made with the fish acclimated to decreasing temperatures with the exception of the hatchery fish that had been acclimated to the fluctuating temperature regime.

Constant temperature bioassays

Median survival times and an incipient lethal level were determined for hatchery and wild cutthroat trout acclimated to 23 and 13-23°C and hatchery trout acclimated to 13-25°C using the method described by Fry (1967) and Brett (1952). Fish were exposed to nominal temperatures of 25.5, 26.0, 26.5, 27.0, 28.0, and 28.5°C (except fish tested in 1975, where 28.0°C was the highest exposure temperature). After placing the fish in the aquaria at the test temperatures, the time of death in minutes and the temperature to the nearest 0.1°C at death were recorded. Each fish was removed at the time of death. Observations were made for 10,000 min (approximately 1 wk) before terminating the experiment. Bioassays on hatchery trout acclimated to 23 and 13-25°C were conducted from 3 July to 27 August 1975. Bioassays on hatchery and wild cutthroat trout acclimated to 23 and 13-25°C were conducted from 18 to 31 August 1976 and were repeated from 13 to 24 September 1976. Probit analysis was used to estimate the MST's and standard errors for each individual bioassay (Bliss 1938; Finney 1971a). The estimates were compared to the original data and a chi-square test was performed for these bioassays to determine if the data were significantly heterogenous (Finney 1971b). The individual MST's were regressed against test temperature for stock x acclimation x replicate runs yielding a total of eight regression lines. An analysis of covariance was made and the slopes of these lines were tested for similarity (Snedecor and Cochran 1967). Adjusted means were then compared using linear contrasts to detect differences in response due to stock, acclimation, or replication (Snedecor and Cochran 1967).

Fluctuating temperature bioassays

Temperature tolerance to fluctuating lethal temperatures of hatchery and wild cutthroat trout acclimated to 23 and 13-23°C were compared. Median cycles to death and incipient lethal level were determined for fish exposed to nominal temperatures of 13-26.5, 13-27.0, 13-27.5, and 13-28.5°C. Tests were initiated in the afternoon when fish acclimated to 13-23°C had reached the peak of the temperature cycle. Ten fish were used for each assay and each test group was introduced into the fluctuating lethal regime at 23°C. The methods used for observing and recording mortality were similar to those described for the constant temperature bioassays. Tests were conducted from 1 to 11 September 1976, and were repeated from 28 September to 4 October 1976.

Once the time of death was converted to cycles and multiplied by 1,000 (to avoid negative logarithms in computations), median cycles to death and standard errors were estimated using the probit technique. The median number of cycles to death for the trout were plotted against the average temperature that the fish were exposed to at peaks of the lethal diel cycle and were compared graphically using 95% confidence intervals to determine if there were any differences between: 1) hatchery and wild fish; 2) fish acclimated to constant or fluctuating temperature regimes; 3) replicates.

Appendix Table 1 contains the experimental design for critical thermal maximum tests, constant and fluctuating temperature bioassays.

RESULTS

Critical Thermal Maximum Determinations

Overall, critical thermal maximums were significantly higher for fish acclimated to constant temperatures than for fish acclimated to fluctuating temperatures during increasing acclimation to 23 and 13-23°C ($t = 7.61$, 146 d.f., $\alpha = 0.05$). No significant differences were detected between hatchery and wild cutthroat trout or between replicates (Appendix Tables 2 and 3 and Fig. 3). The above comparisons were made using linear contrasts of adjusted means of the eight regression lines of CTM x acclimation temperature (AT) for stock x acclimation type x replicate combinations. Although there were no significant differences in the slopes of the regression lines, there was a significant level of heterogeneity of variances between treatments ($X^2 = 26.5$, 7 d.f., $\alpha = 0.05$). In addition, one bioassay out of the 32 was discarded to achieve equality among slopes. The bioassay mentioned had an abnormally low value attributable to only one aberrant individual which significantly altered the regression. The regressions of CTM x AT for hatchery and wild cutthroat trout replicates were pooled to make the two regression equations for fish acclimated to constant and fluctuating temperatures.

The responses of fish acclimated to decreasing temperatures were less easily resolved. This may have been due, in part, to difficulties in controlling the temperature regimes during decreasing acclimation. In addition, some groups were not available for com-

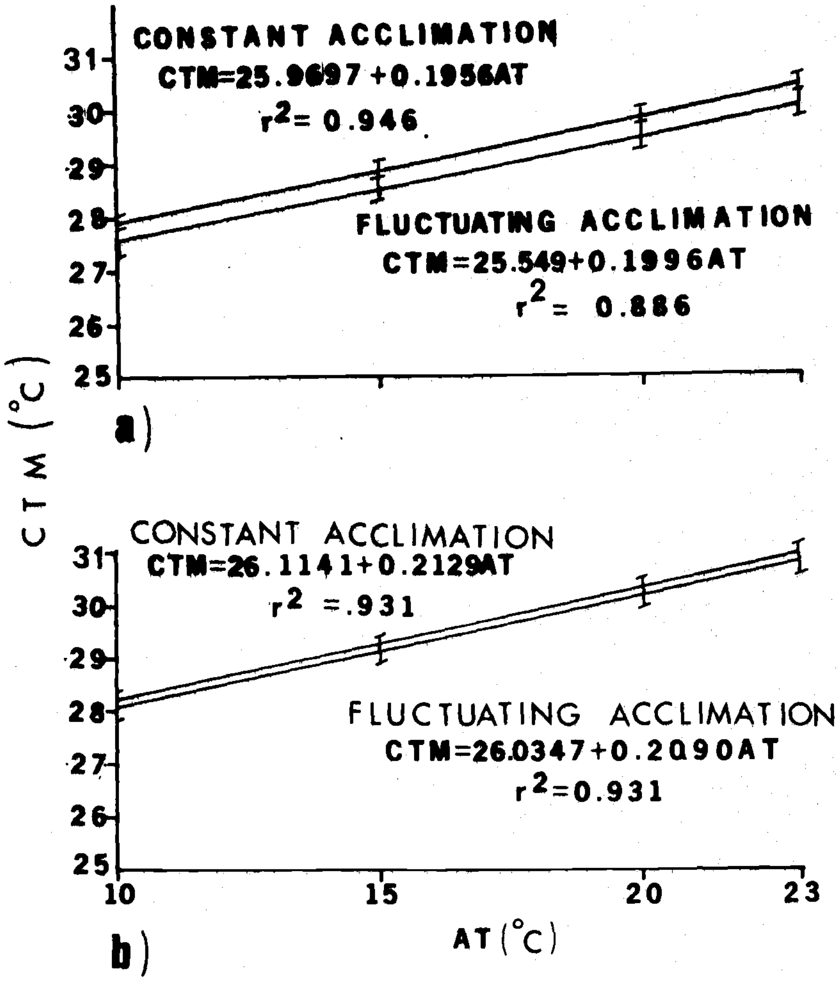

Figure 3. Regressions of critical thermal maximum (CTM) against acclimation temperature (AT) for hatchery, wild or both hatchery and wild cutthroat trout.

(a) Direction of acclimation is from left to right, or increasing. AT refers to acclimation temperature for fish acclimated to constant temperature, or the peak temperature for fish acclimated to fluctuating temperatures. At 15°C for example, fish exposed to fluctuating temperature regime would be acclimated to 8.5-15°C nominal temperatures. Hatchery, wild, and replicates were pooled to make two regressions. Vertical bars indicate 95% confidence limits.

(b) Direction of acclimation is from right to left, or decreasing. Hatchery, wild, and replicates were combined to make the regression for fish acclimated to the constant temperatures. Replicates for the regressions done on wild fish were combined to make a single regression for the fish acclimated to fluctuating temperatures.

parison. The hatchery trout acclimated to 13-23°C were lost when a loose standpipe lowered the tank's water level. Also, one of the the replicates of wild fish acclimated to fluctuating temperatures was discarded because observation was not possible at the time that the CTM was reached.

There was a significant difference in replicates of hatchery and wild fish acclimated to constant temperatures, during decreasing acclimation. Using a linear contrast of the adjusted means of CTM x AT regressions, the first replicates had significantly higher CTM's than the second replicates ($t = 2.51$, 75 c.f., $\alpha = 0.05$). The fact that the CTM's were conducted over a 2 day period with the first replicates on 1 day and the second replicates the following day, might account for such differences. It is conceivable that one more day of acclimation may have contributed to lower CTM values the second day.

When the regressions of wild fish acclimated to constant temperatures were compared to wild fish acclimated to fluctuating temperatures, there was a significant difference in adjusted means, with the former having significantly higher CTM's ($t = 5.33$, 70 d.f., $\alpha = 0.05$). No significant differences were detected between adjusted means of replicates.

Finally, regressions of hatchery and wild fish acclimated to constant temperatures were compared to see if there was a difference in the response to acclimating to warmer temperatures versus acclimation to decreasing temperatures. There was a significant difference in the slopes of the regressions of CTM x AT for fish acclimated to increasing temperatures when compared to fish acclimated to decreasing temperatures ($F = 2.89$, 7, 144 d.f., $\alpha = 0.05$). Fish exposed to decreasing temperatures had a slightly higher rate of decrease in CTM values with decrease in AT, when compared to fish acclimated to increasing temperatures, mainly due to the differences in slope of the regressions. CTM's at each test temperature were higher for fish exposed to the decreasing regime when they were compared to CTM's of fish acclimating to increasing temperatures. More replications of CTM's could have improved the regressions and made interpretation easier.

Constant Temperature Bioassays - 1975 Experiments

Using probit analysis, I estimated the median survival time of hatchery cutthroat trout acclimated to 23 and 13-25°C that were exposed to constant lethal temperatures. MST estimates were made on bioassays that had 80% or more deaths. Fish acclimated to 13-25°C had significantly higher MST's than fish acclimated to 23°C ($F = 7.62$, 1, 5 d.f., $\alpha = 0.05$). The incipient lethal levels were 25.5 and 26.0°C for the fish acclimated to 23 and 13-25°C, respectively (Fig. 4b). Since there was a significant difference between the slopes of the two regression lines of MST against exposure temperature, comparisons of the elevations of these lines (i.e., via linear contrasts of adjusted means) violates conventional bioassay technique (Finney 1971b).

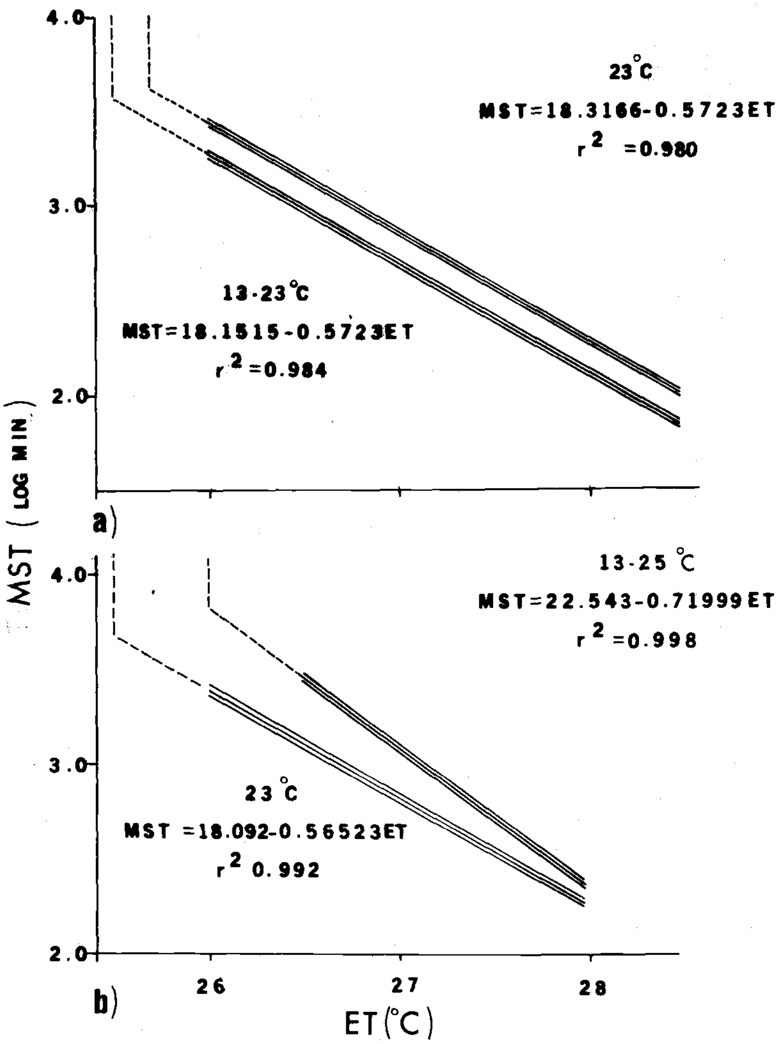

Figure 4. Regressions of median survival time (MST) in log minutes (Log Min) against exposure temperature (ET) in degrees C. The 95% confidence intervals are shown as solid lines on either side of regressions. Vertical dashed lines represent extensions of regression lines.

(a) MST x ET regressions from bioassays of hatchery and wild cutthroat trout acclimated to 23 and 13-23°C, exposed to constant lethal temperatures.

(b) MST x ET regressions of hatchery cutthroat trout acclimated to 23 and 13-25°C.

Constant Temperature Bioassays - 1976 Experiments

The median survival times of fish acclimated to 23°C were significantly higher than those of fish acclimated to 13-23°C. No significant differences were detected between the MST's of hatchery and wild fish, nor were there any differences between bioassays run earlier and those run later in the summer. The above comparisons were made using linear contrasts of the adjusted means generated from the eight regression lines of MST by test temperature for acclimation x stock x replication combinations (Appendix Table 4). Forty seven bioassays where 80% or more mortality occurred were used in making the regressions. There was no significant heterogeneity detected at the 95% level of significance (X^2 = 7.32, 7 d.f., α = 0.05), when individual mortality records were compared to the probit x log survival time regression lines, using the chi-square test (Finney 1971a). Before the analysis of covariance was done, the residual variances of each regression were compared using Bartlett's test. The data were acceptably homogenous at the 95% level of significance (X^2 = 7.32, 7 d.f.). No significant differences were detected between the slopes of the eight regression lines. Regressions of MST by test temperature for hatchery and wild stocks and replicates were pooled together to make two regressions which may be used in predicting MST for a given lethal temperature for cutthroat trout acclimated to 23 and 13-23°C (Fig. 4a). Incipient lethal levels were 25.5 and 25.7°C for fish acclimated to 13-23 and 23°C, respectively.

Fluctuating Temperature Bioassays

Estimates of the median number of cycles to death were made on the groups of fish for the 22 bioassays of fish exposed to a range of fluctuating temperatures where 80% or more fish died. The probit method previously described was used in making the estimates. The estimates of median cycles to death and the 95% confidence limits were plotted and compared in Fig. 5. Hatchery and wild trout acclimated to 23 and 13-23°C had an incipient lethal level occurring at fluctuating temperatures above 13-27.0°C diurnal temperature variation, indicating that the fish would tolerate limited daily exposure to lethal temperatures, over a period of one week. Even with the few estimates obtained, it was obvious that the median cycles to death decreased with an increase in average exposure temperature (measured above 27.0°C). In most cases fish acclimated to 23°C had significantly higher median cycles to death (i.e. longer survival) than fish acclimated to 13-23°C. With the exception of fish acclimated to 23°C that were exposed to an average peak temperature of 27.8°C, there were no significant differences between hatchery and wild cutthroat trout. No clear trend in any possible seasonal shift in temperature tolerance to fluctuating lethal temperatures was apparent. There may have been an upward shift in the incipient lethal level of hatchery and wild cutthroat trout acclimated to 23°C because 90 and 80% mortality occurred for hatchery and wild cutthroat, respectively, in the first period of testing, but only 50 and 70%, respectively, in a later replication.

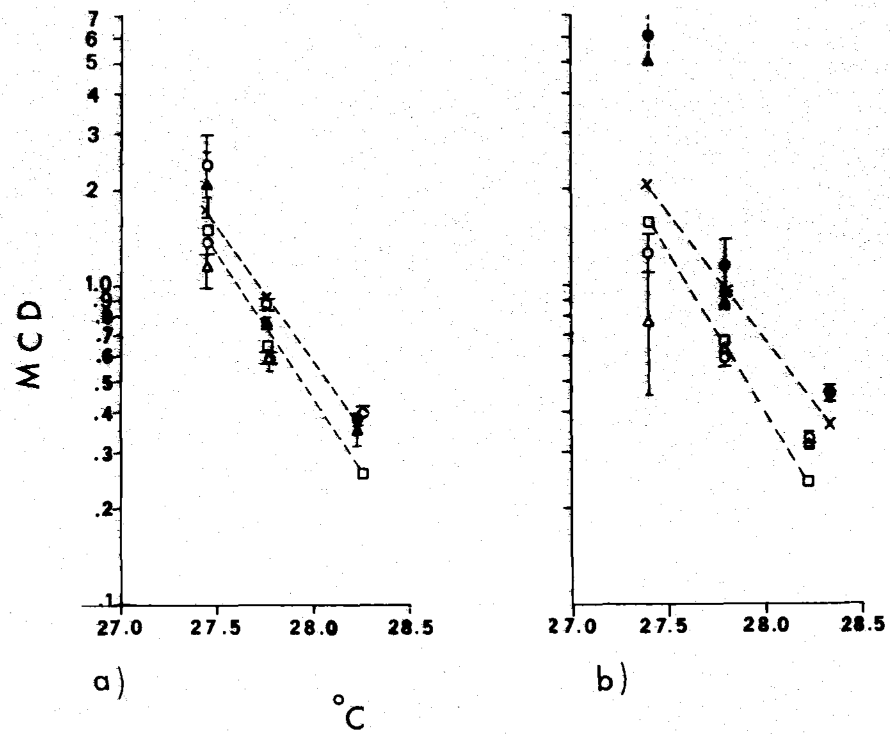

Figure 5. Median cycles to death (MCD) against average exposure temperature above 27.0°C (ET) for hatchery and wild cutthroat trout acclimated to 23 and 13-23°C that were exposed to fluctuating lethal temperatures. Vertical bars indicate 95% confidence limits. Nominal exposure temperatures were 13-27.5, 13-28.0, and 13-28.5°C. Circles represent hatchery and triangles wild fish. Open symbols indicate MCD's of fish acclimated to 13-23°C and solid symbols indicate MCD's of fish acclimated to 23°C. Predicted MCD's are represented by X's for fish acclimated to 23°C and open squares for fish acclimated to 13-23°C. Dashed lines were fitted by eye to the predicted MCD's; (a) Replicate 1, (b) Replicate 2.

DISCUSSION

It is significant that cutthroat trout acclimated to 13-23°C and fish acclimated to 23°C responded similarly to acclimation (measured by change in CTM), constant lethal temperatures and fluctuating lethal temperatures. Rates of change in CTM were about 2°C for every 10°C change in acclimation temperature for fish acclimated to both constant and fluctuating temperatures under both increasing and decreasing acclimation. Fish acclimated to 13-23°C responded to constant lethal temperatures as if they had been acclimated to a constant temperature slighly below the peak but above the mean temperature of the diel cycle. Preliminary experiments conducted in 1974 showed the median survival times of hatchery and wild fish acclimated to 13-23°C were similar to hatchery cutthroat trout acclimated to 20°C. Similar responses to constant and fluctuating types of acclimation were observed in growth and mortality rates of rainbow trout (*Salmo gairdneri*) (Hokanson et al. 1977).

To minimize the cost involved in obtaining information on the lethal tolerance limits of fish acclimated to fluctuating temperatures, resource managers may be able to prescribe an equivalent constant acclimation level to fish acclimated to fluctuating temperatures, that is midway between the mean peak temperatures. In this way, the potential lethal tolerance of fish acclimated to fluctuating temperatures could be assessed utilizing existing constant temperature bioassay information. This relationship may hold until the peak temperatures of fluctuating temperature regimes reach levels equivalent to the highest constant temperature to which fish may be acclimated without significant mortality. This level is about 23°C for cutthroat trout. Higher temperature tolerances may be achieved by acclimating fish to diel cycles with peak temperatures beyond this point. Hatchery cutthroat trout in the 1975 experiments were able to acclimate to 13-25°C with negligable mortality. Although the regression of MST x Test Temperature had a significantly different slope, the fish acclimated to 13-25°C had significantly higher MST's at all test temperatures than did fish acclimated to 23 or 13-23°C.

Differences which occur between the rate of change of median survival times with increasing dosage (in this case, increasing temperature) of two treatment groups usually indicates a biological process that occurs in one treatment group that does not occur in the other (Finney 1971b). In this case the physiological or biochemical processes leading to thermal resistance may be subtly altered for fish acclimated to 13-25°C than for fish acclimated to 23°C.

Otto (1974) also reported an increase in the heat tolerance of mosquito fish (*Gambusia affinis*) acclimated to fluctuating temperature regimes with peak temperatures near or above the incipient lethal level (35 to 50°C), when compared to fish acclimated to constant temperatures (35°C). Greenthroat darter larvae (*Etheostoma lepidum*) had increased resistance to thermal shock under fluctuating temperature regimes when compared to larvae acclimated to constant temperatures (Hubbs 1964). It was also demonstrated by Feldmeth and

Stone (1974) that fluctuating temperatures ranging from 15 to 35°C, increased the scope for thermal tolerance in the desert pupfish (*Cyprinodon nevadensis amargosae*). The fluctuating temperature bioassays reported here indicate that hatchery and wild cutthroat trout could withstand excursions into lethal temperatures as long as the time spent at the peak temperature (27.0°C or above) did not exceed ½ of the median survival time under a constant lethal exposure at the same level.

For example, the MST for fish acclimated to 13-23°C exposed to 27.0°C was 500 min. Fish from the same acclimation temperature that were exposed to 13-27.0°C spent 210 min at 27.0°C and survived the 7 day test period.

Considering the above data, I determined whether or not present water quality criteria would be sufficient to protect 0-age hatchery and wild coastal cutthroat trout, when these criteria are applied to a temperature regime that simulates a post-logging situation. Water quality criteria have been established for aquatic life that may be exposed to either short or long duration temperature increases above ambient stream temperature (U.S.E.P.A. 1973). I only considered the criteria for extreme temperature exposures of short duration. If:

$$1 \geq \frac{\text{Time}}{10^{(a + b\{\text{temp.} + 2\})}}$$

where: Time = time spent at the lethal temperature in min

Temp. = exposure temperature in °C

a = intercept of the MST x temperature regression

b = slope of the MST x temperature regression

c = $2^{\circ}C$ safety factor

then the criteria have been met, and the safety of the fish (i.e., no mortality) is considered to be insured. The regression equation is MST = 18.1515 - 0.5723 ET for fish acclimated to 13-23°C. The 13-27.0°C regime, which was near the threshold of mortality for fish acclimated to 13-23°C, had an exposure time of 210 min at 27.0°C. When the exposure time and the regression equation, with the 2°C safety factor, was substituted into the criteria formula, the inequality 1 < 5.85 was obtained and the criteria not met. Since no mortality was observed at 13-27.0°C, the criteria would be sufficient to protect the fish. If the criteria formula is solved for the equality

$$1 = \frac{210 \text{ min.}}{10^{(18.1515 + \{-\}\{0.5723\}\{ET + 2\})}}$$

the highest temperature that the fish acclimated to 13-23°C could be exposed to would be 13-25.6°C without violating the criteria for short term exposures (over a period of one week or less). The foregoing conclusions must retain the constraints that the simulated regime was produced in the laboratory and not under strictly natural conditions.

The above criteria aid in determining safe levels of temperature exposure for fish. In some cases the resource manager may want to actually predict mortality that might occur under more extreme conditions. One predictive model has been used to forecast death of juvenile coho salmon (*Oncorhynchus kisutch*) exposed to fluctuating lethal temperatures. DeHart (1974) conducted constant temperature bioassays on fish acclimated to 20°C and used the MST's to predict mortality of fish from the same acclimation regime that were exposed to fluctuating lethal temperatures. The estimated median cycles to death (MCD) was based on the inverse of the sum of individual fractions of median survival time spent at each 0.25°C increment above the incipient lethal level. I determined whether or not a similar technique could be applied to the fluctuating temperature bioassays reported in this paper. Using the formula:

$$MCD \doteq \frac{1}{\sum_{I}^{T} 6f/MST_{I}}$$

*NOTE: ≐ means approximately equal to.

where:

MCD = median cycles to death

f = the number of 6 minute intervals at a given temperature level, taken to the nearest 0.1°C.

MST = the median survival time based on the linear regression equations in Fig. 4a.

I = the initial temperature increment or 27.0°C

T = the final temperature increment

Estimates of MCD were made for cutthroat trout acclimated to 23 and 13-23°C and were plotted in Figures 5a and b. In both DeHart's model and in my modified version the estimated MCD can only be approximated by the equation, since what is measured is the resistance of time of fish exposed to a lethal cycle and not the median survival time. If resistance time is assumed to be strictly accumulative, then a more realistic estimator might use the regression equation of individual resistance times against exposure temperature. In any event, the estimator used here provided estimated MCD's that approximated reality in many cases (see Fig. 4). Since no confidence limits could be calculated for these estimates, no statistical comparisons could be made.

The water quality criteria have been shown to be adequate enough for wide application and provide ample protection for coastal cutthroat trout acclimated to temperature regimes simulating post-logging conditions. The selection of an appropriate acclimation temperature for fish acclimated to fluctuating temperature regimes presents no problem if the resource manager uses constraint. With the ability to predict stream temperature (Brown 1969) for post-logging situations (Brown 1970), the manager can examine hypothetical temperature regimes that might occur under different timber removal and slash burning schemes. To establish the limits for thermal tolerance under fluctuating temperature regimes the resource manager would then have to select an acclimation level for fish (in this case, cutthroat trout) that would correspond to the hypothetical temperature regime. If the rate of acclimation was similar to that carried out in these experiments, a one week average of the temperatures midway between the mean and peak temperatures should suffice as an equivalent constant acclimation temperature for cutthroat and the upper tolerance limits determined.

Where the relationship of MST versus ET yield similar rates of change between fish acclimated to constant and fluctuating temperatures, and alternative method for finding an equivalent constant level of acclimation for fish acclimated to dielly cycling temperatures may exist. For the fish acclimated to 23°C and 13-23°C in this experiment, the regressions of MST versus ET are functionally related and may be solved for one another. Since the slopes of the two regressions are the same, MST's for fish acclimated to 13-23°C can be expressed as: MST = (18.3166 - 0.5723 ET) - 0.1641. The portion of the equation in the parenthesis is taken from the right hand part of the linear regression equation for fish acclimated to 23°C (see Fig. 4). Care must be taken to make the subtractions on the right side of the equation before taking the antilog to find the time in min.

More experimental work would be necessary to see if the same relationships holds for other diel cycles with different peak temperatures. If it did, 0.1651 could be subtracted from the regression equation of MST versus ET of fish acclimated to the constant temperature that was equal to the peak of the diel cycle. Further work should include the investigation of the effect of the range of the diel cycle on the above transformation.

In the event that preventative steps are not taken during logging and slashburning, and severe environmental degradation leads to a fish kill from thermal effects, the resource manager can assess the kill using the predictive models described in the second example, providing that he has a temperature record of the event and thermal tolerance information on the fish involved. But, these considerations do not account for the potential effects of other environmental parameters on the lethal tolerance of fish.

SUMMARY

1. There were no significant differences in thermal tolerance between hatchery and wild cutthroat trout.
2. Constant temperature bioassay information could be used to predict the mortality of coastal cutthroat trout exposed to lethal fluctuating temperatures.
3. Water Quality Criteria for short term exposures to lethal temperatures were sufficient for protecting coastal cutthroat trout acclimated to a temperature regime that simulated a post-logging condition in the laboratory. For fish acclimated to 13-23°C violation of the Criteria occurs at 13-26.0°C.
4. Coastal cutthroat trout can withstand exposures to large diel cycles up to 13-27.0°C daily variation with 10% or less mortality over a 1 wk period (7 cycles). Survival occurs if the time spent at the peak temperature is less than approximately ½ the MST of the fish when exposed to a constant temperature equal to the peak temperature of the diel cycle.
5. Selecting the temperature that is midway between the mean and the peak temperature of a diel cycle may yield an approximate constant temperature acclimation level for fish acclimated to a fluctuating temperature regime.

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Appendix Table 1. Experimental design for critical thermal maximum tests (CTM), constant temperature bioassays (CT) and fluctuating temperature bioassays (F) conducted during the summers of 1975 and 1976. Each test for CT or F given in the table was conducted on 10 fish, and every test of CTM employed two replicates of 5 fish each. Each CT conducted in 1975 represents two replicates of 10 fish each. "Warming" refers to the gradual increase in temperature to summer time acclimation temperatures and "cooling" refers to the gradual decrease in temperature back to winter time (or pre-logging) acclimation temperatures. See text for complete description of experiment. H and W indicate where tests were conducted using hatchery and wild cutthroat trout, respectively.

Acclimation Temperature (°C)		Summer Acclimation Temperatures					
		1975		1976			
		23°C	13-25°C	23°C		13-23°C	
Constant	Fluctuating	Hatchery Only		H	W	H	W
10	7.5-10			CTM	CTM	CTM	CTM
15	8.5-15			CTM	CTM	CTM	CTM
20	10-20			CTM	CTM	CTM	CTM
23	13-23			CTM	CTM	CTM	CTM
Early Summer		CT	CT	CT,F	CT,F	CT,F	CT,F
Late Summer		CT	CT	CT,F	CT,F	CT,F	CT,F
23	13-23			CTM	CTM	--	CTM
20	10-20			CTM	CTM	--	CTM
15	8.5-15			CTM	CTM	--	CTM
10	7.5-10			CTM	CTM	--	CTM

Appendix Table 2. Mean critical thermal maximum temperatures (°C) achieved by hatchery and wild cutthroat trout acclimated to constant and fluctuating temperatures during acclimation to increasing and decreasing temperatures. Nominal acclimation temperatures (°C) are listed. Each X was calculated from the combination of two replicates, the combined n=10.

Temperature regime	Acclimation temperature °C	Hatchery X	Hatchery S.E.	Wild X	Wild S.E.
Increasing	10	28.03	0.09	27.98	0.09
	15	28.88	0.07	28.90	0.07
	20	30.05	0.11	29.90	0.11
	23	30.62	0.10	30.57	0.10
	7.5-10	27.64	0.09	27.68	0.09
	8.5-15	28.54	0.07	28.32	0.07
	10-20	29.52	0.11	29.50	0.11
	13-23	30.31	0.10	31.20 a/	0.10
Decreasing	23	30.69	0.09	30.90	0.09
	20	30.33	0.08	30.36	0.08
	15	29.45	0.06	29.48	0.06
	10	28.05	0.09	28.23	0.09
	13-23	-- b/		30.56	0.10
	10-20	--		29.82	0.08
	8.5-15	--		29.38	0.06
	7.5-10	--		28.02	0.08

a/ *In these cases, n=5 as only one of replicate was used.*

b/ *The hatchery fish acclimated to fluctuating temperatures were lost prior to acclimation to lower temperatures.*

Appendix Table 3. Regression equations of critical thermal maximum (CTM) in degrees C against acclimation temperature (AT) in degrees C, and r^2 for the treatments compared in the analysis of covariance. Hatchery (H) and wild (W) cutthroat trout acclimated to both increasing (I) and decreasing (D) constant (C) and fluctuating (F) temperature regimes were compared, with the exception of the hatchery fish that were lost prior to decreasing fluctuating temperatures. Replicates are indicated as 1 or 2 under treatment.

Treatment	Regression equation	r^2
HCI1	CTM = 25.978 - 0.19165AT	0.929
HCI2	CTM = 25.877 - 0.20677AT	0.977
WCI1	CTM = 26.037 - 0.18617AT	0.983
WCI2	CTM = 25.984 - 0.19805AT	0.966
HFI1	CTM = 25.110 - 0.22506AT	0.925
HFI2	CTM = 25.912 - 0.18647AT	0.810
WFI1	CTM = 25.972 - 0.14602AT	0.918
WFI2	CTM = 25.332 - 0.20369AT	0.986
HCD1	CTM = 26.508 - 0.18582AT	0.911
HCD2	CTM = 25.717 - 0.23289AT	0.918
WCD1	CTM = 26.042 - 0.21274AT	0.959
WCD2	CTM = 26.183 - 0.22053AT	0.961
WFD1	CTM = 25.519 - 0.23174AT	0.942
WFD2	CTM = 26.354 - 0.18504AT	0.943

Appendix Table 4. Regression equations of median survival time (MST) in logmin against exposure temperature (ET) in degrees C and r^2 for individual treatment groups that were compared in the analysis of covariance. The treatment groupings were made up of 1976 data collected on hatchery (H) and wild (W) cutthroat trout acclimated to 23°C (C) and 13-23°C (F) during the constant temperature bioassay trials. Replicates are indicated as 1 and 2 under treatment.

Treatment	Regression equation	r^2
CH1	MST = 18.8858 - 0.59159ET	0.994
CH2	MST = 17.2450 - 0.53392ET	0.989
CW1	MST = 18.471 - 0.57769ET	0.991
CW2	MST = 18.616 - 0.58361ET	0.954
FH1	MST = 18.036 - 0.56783ET	0.979
FH2	MST = 17.902 - 0.56298ET	0.984
FW1	MST = 18.386 - 0.58212ET	0.996
FW2	MST = 17.927 - 0.56324ET	0.980