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An Evaluation of Stress Induced Mortality of Stocked Catchable-Sized Rainbow Trout in Temple Fork of the Logan River

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AN EVALUATION OF STRESS INDUCED MORTALITY OF STOCKED CATCHABLE-SIZED RAINBOW TROUT IN TEMPLE FORK OF THE LOGAN RIVER

by

Robert Earl Gresswell

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by

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The level of stress imposed by population pressure, handling and live transportation on planted catchable-sized rainbow trout in a northern Utah stream was examined. Production of adrenocorticotrophin, as measured by interrenal ascorbic acid and serum cortisol levels, did not occur in transported or planted fish.

Dead or moribund fish collected from stream or live boxes comprised 13 percent of the 2,000 fish planted. Aeromonas salmonicida, the causative agent of furunculosis, was isolated from 41 percent of 106 moribund fish sampled. In addition, 39 percent of the samples exhibited bacterial growth other than A. salmonicida. Apparently, handling, transportation, and planting did cause low levels of stress sufficient enough to induce stress-mediated diseases such as furunculosis.

(35 pages)

INTRODUCTION

Although the planting of catchable-sized fish has been regarded by some as a modern innovation, the practice of raising trout to a larger size (130-160 mm) before stocking was recognized as early as 1886 to yield superior results when compared to fingerling plants. It was not until the late 1920's, however, that this principle gained acceptance. Since that time, the practice of stocking catchable-sized trout has evolved into general policy (Wood, 1953).

It has been estimated that numbers of sport fisherman will increase 50 percent by 1976 and 150 percent by 2000 over 1960 figures (U.S. Dept. of the Interior, 1962). Stroud (1970) suggested this will result in 63 million anglers fishing 1.3 billion days by the year 2000. Despite the great controversy surrounding the policy of stocking catchable-sized trout, it remains a primary management tool to meet the increasing fishing pressure throughout the country (Schuck, 1948; Calhoun, 1966; Little, 1966; Locke, 1966; Seaman, 1966).

Results from the stocking of catchable-sized trout reveal the need for improvement; nationwide estimates of fish return are approximately 50 percent (Meigs and Mullenbach, 1953; Needham, 1959; Calhoun, 1966; Locke, 1966). If fish managers are to meet the estimated increases in fishing pressure through the use of planted catchables, the number of fish required, given current returns, would be phenomenal. An increase in the survival of catchable-sized trout after planting, however, would lessen the burden of hatchery production considerably (Kramer, 1969).

In the past two decades, there has been an increase in research on the causes of mortality of catchable-sized trout with special emphasis on delayed mortality or deaths occurring 1 to 7 days after planting (Horton, 1956). Miller (1953, 1955, 1958) reported that competition between resident wild trout and hatchery trout attributed significantly to this delayed mortality. Competition for both food and space soon after stocking forced the hatchery fish to remain in the stronger current. This constant struggle to maintain position caused an increase in blood lactate resulting in death of weaker fish. Although the actual cause of death could not be established, Miller hypothesized that it could be general acidosis or the reduction of some essential metabolite exceeding the capacity for replacement.

Black (1955, 1956, 1957) stated that a severe disturbance of the acid-base balance due to accumulation of lactic acid in the blood stream may be a principal cause of delayed mortality in planted hatchery trout. Hyperactivity due to forced exertion in a current, handling, or live transportation was stated as a possible cause of fatigue and the accumulation of metabolic by-products.

Miller, Sinclair and Hochachka (1959) reported a high correlation between hatchery diet and glycogen stores. Hochachka and Sinclair (1962) concluded that a reduction of these metabolic reserves may be a cause of delayed mortalities. In addition to the effects of transportation methods, temperature, and accumulations of lactic acid, they suggested that the reduction of an essential metabolite such as glycogen, in conjunction with an increased metabolic rate, may be a significant cause of mortality in planted hatchery trout.

Although empty stomachs in dead fish recovered after stocking have been recorded (Miller, 1951; Hochachka and Sinclair, 1962), it appears doubtful that starvation is involved in delayed mortalities, since rainbow trout have been reported to survive 248 days without food (Anonymous, 1956). Reimers (1957) and Adelman, Bingham and Maatch (1955) further support the idea that starvation is not a critical factor in the mortality of catchable-sized trout.

The ability of a fish to utilize oxygen is critical to the accumulation of lactic acid in the blood. Under conditions of extreme exercise, such as maintaining position in a strong current, stored glycogen is metabolized into glucose. The glucose is then catabolized to yield pyruvate, resulting in a release of energy and hydrogen. In the presence of oxygen, hydrogen is oxidized, and the pyruvate continues to break down releasing more energy. In the absence of oxygen, however, hydrogen is bound to the pyruvate and lactic acid is formed (Black, 1958). As mentioned previously, this buildup of lactic acid is thought to play a major role in delayed mortality of planted trout.

The maximum metabolic (active) rate of oxygen consumption in fish is limited by the dissolved oxygen concentration of the water, water temperature, blood hemoglobin, and many other factors. The oxygen utilized for normal body maintenance is termed the standard metabolic rate; the difference between the active and standard rates has been defined as scope-for-activity (Fry, 1947). Since the maximum rate for given conditions is fixed, a lowering of the standard rate would increase the scope-for-activity (Dickson and Kramer, 1971).

Since 1965, the Utah Cooperative Fish Unit has been exploring the use of scope-for-activity as an index to the survival of catchable-

sized hatchery trout. Dickson and Kramer (1971) and Dwyer and Kramer (1973) found water temperature to be an important factor influencing scope-for-activity. They also reported that wild rainbow trout had a higher scope at 25 C than did domestic strains. Scope-for-activity was highest after six days starvation. In a field test of these results, Bricker (1970) found in high gradient sections of the stream that fish starved for six days returned to the creel in greater numbers than one-day starved fish (standard hatchery procedure).

Research conducted thus far has been based on the general assumption that delayed mortality of catchable-sized trout was due indirectly to some form of environmental stress. Brett (1958) defined stress as:

> A state produced by any environmental or other factor which extends the adaptive responses of an animal beyond the normal range, or which disturbs the normal functioning to such an extent that, in either case, the chances of survival are significantly reduced.

The ability to evaluate conditions within the environment which may invoke stress upon fish would be invaluable. The use of biochemical and physiological changes in fish as a result of stress has been investigated (Hane et al., 1966; Faggerlund, 1967; Wedemeyer, 1969a, 1969b, 1970, 1972; Wedemeyer and Chatterton, 1970; Wedemeyer, Ross and Smith, 1968). Although relationships have been developed in the laboratory, no work has been published on field evaluations of these indices.

Objectives

If field evaluations of methods to increase survival of hatchery trout in Utah are to be useful, the causes and extent of mortality in Utah streams must first be determined. Because of its location, size

and abundant food organisms, Temple Fork of the Logan River has been selected by the Utah Cooperative Fishery Unit as an experimental stream. Thus, it would be advantageous to investigate the causes of mortality of planted trout in this stream. Temple Fork is typical of many Utah streams, so this information would be important for the entire state.

The principle objective was to determine the level of stress imposed by population pressure, handling and live transportation on stocked catachable-sized rainbow trout in Temple Fork of the Logan River.

STUDY AREA

Temple Fork of the Logan River is a small mountain stream in northeastern Utah. Description of the area is given by Pearson and Kramer (1972). Temple Fork flows 7.3 km from its source at 1,990 m above sea level to its confluence with the Logan River. This point is approximately 32 km from the city of Logan. A dirt road runs parallel to the stream for the first 5.6 km above the mouth, but the upper 1.7 km are accessible only by foot.

The study was confined to a 500 m section located 4.6 km above the mouth of Temple Fork. Mean gradient for this section was 35 m/km and mean width 3.8 m. This section of stream flowed west through a canyon which gradually widened into open flats, and was characterized by riffle areas, with a few large pools. The streambed was composed of loosely associated sand, gravel and some large rubble. Aquatic mosses (Musci) were common on partially submerged rocks. The stream was shaded by aspen (Populus tremuloides), chokecherry (Prunus sp.), willows (Salix spp.) and wild rose (Rosa sp.) with intermittent sections of bluegrass (Poa sp.) along the stream (Pearson and Kramer, 1972).

Food organisms were abundant. Pearson (1970) reported a mean monthly number of all macroinvertebrates of 1,688/0.1 m^2 in this area of Temple Fork. Most abundant macroinvertebrate forms were Ephemerella sp., Oligophlebodes sigma, Baetis bicaudatus, Rhithtogena, Cinygmula, Chironomidae, Tricladida, Glossosoma, Tipulidae, Nemoura, Elimdae, and Psychondidae.

Wild populations of brown trout (Salmo trutta) and cutthroat trout (S. clarki) were present in Temple Fork, and catchable-sized rainbow trout (S. gairdneri) have been stocked several times each summer. Angling pressure was heavy during the early fishing season and on holiday weekends but light during the remainder of the season (Pearson, 1970).

METHODS AND MATERIALS

Measurement of Physical Parameters

Stream temperatures were monitored by a Foxboro 30-day continuous recording thermometer, located 25 m above the culvert (Figure 1). A hand thermometer was used to measure water temperature of the hatchery raceways, the hatchery truck and the stream at the time of stocking.

Stream discharge was measured at the upstream end of a large culvert located immediately below the study section (Figure 1). A Gurley current meter was used to measure daily stream velocity at the culvert entrance. The average of two velocity readings was used to compute total discharge from the culvert.

Capture Methods

Prior to the study, resident fish were removed by electrofishing from the study section. Captured fish were released downstream from the culvert. The 500 m study section of Temple Fork was closed to sport angling during the entire 1972 fishing season.

A fish trap was placed in the stream at the lower boundary of the study section and anchored with steel cable. It consisted of two 6 m wings and a trap box made of heavy wire screen. Each wing was constructed of aluminum conduit 3.8 cm in diameter. Ten sections of conduit, spaced 1.9 cm apart, were used yielding a height of 56 cm for each wing. The trap box included a door for fish removal and an entrance funnel to prevent escape. The trap box was secured to the wings by eye hooks (Twedt, 1973).

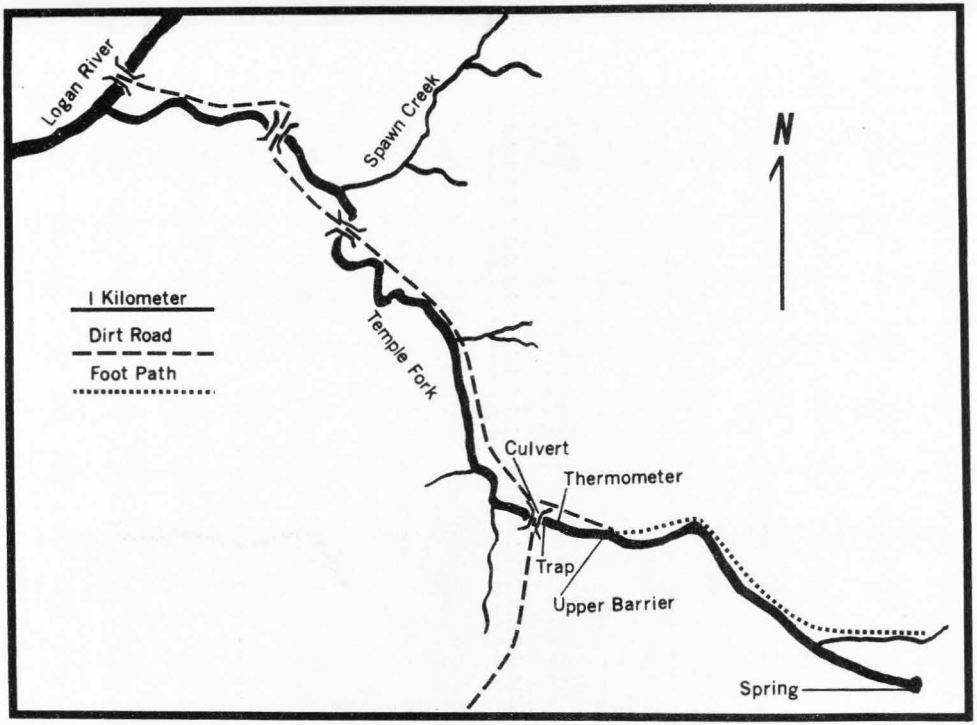

Figure 1. Map of Temple Fork showing the location of culvert, recording thermometer, trap, and upper barrier in the study section.

A fish counter[1] was modified by Twedt (1973) to sound an alarm whenever a fish entered the trap. Two tunnels, one "dummy" and one "live," were connected side by side to the trap box passed through the "live" tunnel, interrupting the electrical field and causing the alarm to ring until manually turned off. Debris or air bubbles did not trigger the alarm.

A 12 m barrier, constructed in a similar manner to the above described trap wings, was placed 500 m above the trap to isolate the study section. The barrier was placed diagonally across the stream to prevent major water back-up.

Fish were collected immediately upon entering the trap and anesthetized in a solution of 20 ppm tricane methanesulfonate (MS-222) and 5 ppm quinaldine (Schoettger and Steucke, Jr., 1970). Lengths in mm and weights in g were recorded. Captured fish were categorized as follows:

1. Dead
2. Unstable: exhibited loss of equilibrium
3. Weak: did not maintain position in the current in the trap but retained equilibrium
4. Stable: appeared normal

Random samples of physiological parameters (see section on physiological indicators of stress) were taken from fish in the latter three categories at the time of capture, and the remaining fish were placed in live boxes for observation.

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[1]Borrowed from National Marine Fisheries Service, Northwest Fisheries Center, Seattle, Washington.

Fish Marking

All fish utilized during the study were catchable-sized (165-334 mm) rainbow trout (Salmo gairdneri) of the Mount Whitney Strain. The trout were supplied by the Logan Production Hatchery, Utah Division of Wildlife Resources. One day prior to tagging, two lots of 400 fish each were transported in a hatchery truck from the Production Hatchery to separate raceways at the Fisheries Experiment Station, Utah Division of Wildlife Resources. Each concrete raceway was 6 m long, .91 m wide and .6 m deep (water depth). Water was piped directly from the flowing well into the raceways at the rate of 0.0014 m^3/sec. Mean water temperature was 17.8 C.

Individuals of one lot were tagged using a green internal anchor tag (Dell, 1968) five days before stocking. The five-digit number on each tag provided individual identification. During experimental periods three, four and five, the tagging gun was immersed in alcohol and distilled water between tagging individuals to reduce the occurrence of lesions at the point of tag insertion (Stobo, 1972).

During the marking process, length in mm and weight in g were recorded for each fish. Fish were anesthetized in a solution of 20 ppm MS-222 and 5 ppm quinaldine to minimize stress from handling. The second lot of 400 fish remained untagged.

Experimental Periods and Treatments

The study was divided into five two-week experimental periods separated by one-week intervals. Fish were planted on the first day

of each period from June to September, 1972 (Table 2). Fish were subjected to three treatments: control, transported and planted.

Trout were held in the raceways and fed according to standard hatchery procedure until the date of stocking. Final feeding was the afternoon of the day before stocking.

Twenty four hours before stocking, 15 fish from the untagged lot were sacrificed for blood pH, ascorbic acid, and serum cortisol determinations. These determinations were used to establish control levels for respective experimental periods.

Lots of 400 tagged rainbow trout were planted at the upper barrier. All fish were planted at 12:00 noon. A 795 l fiberglass coated tank (E. McLeary, Soap Lake, Washington) aerated with bottled oxygen was used for transportation of fish to planting site. In cases where the difference between the stream and hauling tank temperature was greater than 3 C, the hauling tank was tempered to within this range before fish were released. Tempering consisted of adding stream water until the desired temperature was attained and involved approximately 30 minutes.

In order to determine the effects of transportation and handling on mortality, 400 fish of the untagged lot were transported in the hauling tank an equal period of time and returned to the hatchery. Mortalities during transportation were recorded, and upon arrival at the hatchery blood pH, ascorbic acid and serum cortisol determinations were made on a sample of 15 fish. The remainder of the fish were returned to the Production Hatchery.

Physiological Indicators of Stress

All fish used for physiological analyses were first anesthesized using a solution of 20 ppm MS-222 and 5 ppm quinaldine.

Interrenal ascorbic acid determinations

Interrenal ascorbic acid analyses were conducted using the method of Polk, Flanagan and Van Loon (1960) as modified by Wedemeyer (1969a). Kidneys weights were measured on a pan balance. Samples weighing between 0.5 - 1.0 g were homogenized with ten volumes of 5 percent HPO_3 in 10 percent acetic acid and filtered. A 2 ml aliquot of the filtrate was used for analysis.

Samples from 15 fish were taken one day prior to each stocking at the hatchery and from transported untagged fish. Random samples were taken from planted fish as they entered the trap and from all fish being held in live boxes which exhibited a moribund condition.

Samples taken in the field were frozen with dry ice immediately after filtration and transported periodically to Utah State University. Ascorbic acid samples obtained at the hatchery were taken directly to USU for freezing. Samples were analyzed within two weeks of sampling date.

Blood pH determinations

Blood samples were taken from anesthetized fish by cardiac puncture using 2 1/2 cc disposable syringes and emptied into 4 ml glass tubes. Since normal blood pH of fish changes with temperature (Wedemeyer and Chatterton, 1971), blood temperature was maintained approximately equal to the water temperature by placing the tube in a beaker

filled with water from which the fish were taken. Water temperature was recorded.

Blood pH was read directly from the tubes using a Chemtrix (420 E) pH meter with a Markson (model 808) combination electrode. During the first experimental period, a Fisher (model 210) pH meter was used with the Markson electrode.

Serum cortisol determinations

After pH measurements were recorded on each blood sample, the serum was separated by centrifugation and frozen. Dry ice was used for field samples. Samples were pooled at the time of analysis to obtain the required amount of serum for the procedure. Samples for cortisol determination were not analysed until December, 1972. Serum cortisol analyses were conducted using the method described by Clark and Rubin (1968).

Disease Observations

All fish in a moribund condition, whether at the time of capture or while being held in a live box, were examined for Aeromonas salmonicida, the causative agent of furunculosis. Fish which exhibited external lesions were sampled regardless of their condition. The rapid invasion of dead fish by numerous types of bacteria prevented sampling of fish after death.

Each fish was anesthetized in a solution of 20 ppm MS-222 and 5 ppm quinaldine. The dorsal portion of the body was dried and wiped with alcohol. Using a sterile blade, a vertical incision was made just anterior of the dorsal fin into the vertebral column. The anterior portion of the fish was bent forward so that a flamed loop could

be inserted into the kidney. The loop was rotated several times, removed and a smear was made on a Furunculosis Agar slant (Griffin, Snieszko and Friddle, 1953). The slants were delivered to the Fisheries Experiment Station for incubation at 22 C. Results were recorded by station staff.

Post-experimental Population

Following the final experimental period, the entire study section was surveyed using a back-pack shocker with a variable voltage pulsator utilizing direct current. Length in mm and weight in g were recorded for each fish captured before returning them to the stream below the fish trap. An estimate of the post-experimental population was made using the multinomial method (Zippin, 1958).

RESULTS

Stream Temperature and Discharge

Average water temperatures during the five experimental periods ranged from 7.2 to 8.2 C (Figure 2). Temperature was at a minimum during the first period, peaked during the third and gradually decreased during the latter periods. In only one instance was the difference between hauling water and stream temperatures greater than 3 C (Table 1). Tempering involved approximately 30 minutes in this case.

Mean stream flows ranged from .63 to .32 m^3/sec (Figure 2). Peak flows occurred during experimental period one and decreased gradually to a minimum in period five. Rate of decrease appeared to be greatest between periods one and three.

Table 1. Raceway, hauling tank and stream temperatures (^oC) at time of stocking for each experimental period, Temple Fork study, 1972.

Period	Raceway	Hauling Tank	Stream
1	17.8	11.1	5.5
2	17.8	5.3	6.7
3	17.8	7.8	6.1
4	17.8	8.3	7.2
5	17.8	7.8	7.2

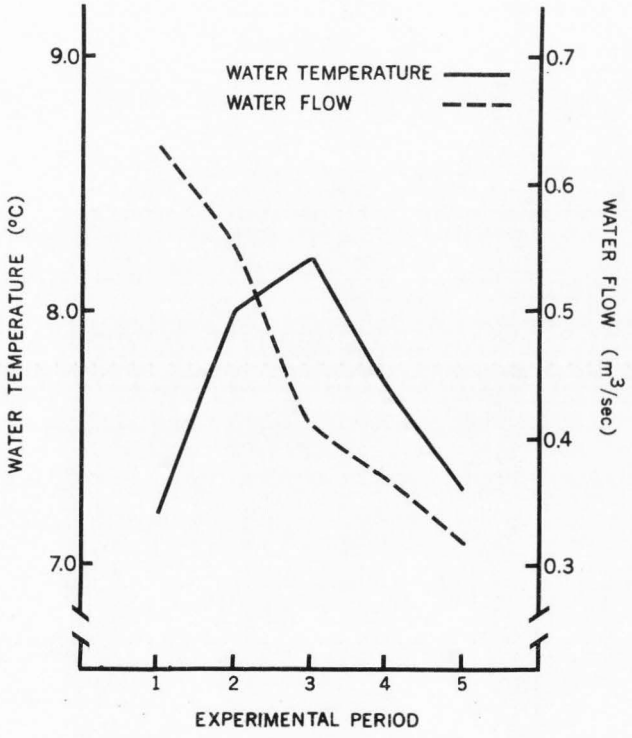

Figure 2. Mean water temperatures and flow rates during each experimental period, Temple Fork study, 1972.

Trap Returns and Mortality Estimates

The number of fish collected from the trap during an experimental period ranged from 62 to 128 (Table 2). Returns from downstream tag boxes and subsequent sampling below the study area indicated that many of the experimental fish bypassed the trap without detection. Apparently, fish slipped between the bars of the trap or through areas where the substrate was eroded from beneath the trap.

Table 2. Number of fish collected in trap during each experimental period, Temple Fork study, 1972.

Period	Dates	Fish Collected	Fish Planted
1	June 14-28	90	398
2	July 5-19	128	392
3	July 26-Aug. 9	62	395
4	Aug. 16-30	100	394
5	Sept. 6-20	130	398

Because of continued growth of hatchery stock throughout the summer, size of fish used for stocking during successive experimental periods was significantly different at the 0.05 level (Table 3). These differences were also reflected by the size of fish caught in the trap during the respective periods. During periods two, four, and five, either length or weight of captured fish was significantly smaller than average measurements of marked fish for the respective experimental periods. Growth, as measured by changes in weight and

length of individual fish from time of marking to time of capture, was not significant ($\alpha = 0.05$, $F_L = 1.70$, $F_W = 0.001$) during any of the five experimental periods.

Table 3. Mean length in mm and weight in g of fish at time of marking and time of capture for each experimental period, Temple Fork study, 1972.

	Length			Weight		
Period	At Marking	At Capture	F	At Marking	At Capture	F
1	241.2	240.9	.013	153.3	150.4	.380
2	256.9	252.8	5.4*	166.7	160.6	3.63
3	248.3	250.3	.724	155.0	152.8	.226
4	264.9	259.9	6.42*	200.0	178.7	27.70**
5	281.6	279.8	.560	240.9	225.8	7.28**
	F=198.2**	F=64.9**		F=312.3**	F=86.1**	

* Significant at the 0.05 level.
** Significant at the 0.05 and 0.01 levels.

Percentage of fish in each category of condition at time of capture varied among experimental periods (Table 4). These differences were not significant ($X^2 = 0.48$) at the 0.05 level. The number of fish sacrificed for physiological samples in each category varied throughout the study, so it was impossible to compare the effect of condition on eventual mortality.

Dead and moribund fish collected during the entire study from either the trap or the live boxes comprised 12.99 percent of the fish

planted. The majority of these (96 percent) were found while being held in the live boxes. The number of fish which died or were found in moribund condition gradually declined from period two through period five (Table 5).

Table 4. Percentage of total fish captured during each experimental period and category of condition, Temple Fork study, 1972.

	Condition			
Period	Stable	Weak	Unstable	Dead
1	.60	.22	.11	.07
2	.63	.34	.02	.01
3	.47	.43	.10	.00
4	.64	.32	.03	.01
5	.78	.17	.03	.02
	$X^2 = .4848$			

Table 5. Number of dead or moribund fish observed during each experimental period and one-week post period interval, Temple Fork study, 1972.

	Dead or Moribund Fish			
Period	During Period	Post Period	From Previous Plantings	Total
1	51	12	0	63
2	42	17	8	67
3	26	9	13	48
4	24	11	10	45
5	22	--	13	35

Causes of Mortality

Physiological indicators of stress

Interrenal ascorbic acid. Determinations of interrenal ascorbic acid were not made during the first experimental period because of problems in sampling technique. For remaining periods, ascorbic acid levels ranged from 11.3 to 387.8 µg/g kidney (Appendix, Table 9). No significant differences ($\alpha = 0.05$) were found among control, transported, and planted groups for levels of ascorbic acid within each experimental period (Table 6).

When results from all experimental periods were analyzed, differences between transported and planted groups were not significant at the 0.05 level. Among control groups, experimental period four differed significantly ($\alpha = 0.05$) from periods three and five.

Table 6. Mean levels of ascorbic acid in µg/g kidney for each treatment, experimental periods two through five, Temple Fork study, 1972.

Period	Control	Transported	Planted	F
2	186.4	147.4	171.5	1.75
3	163.6	165.9	185.9	.76
4	212.4	213.4	183.4	1.36
5	158.3	178.3	168.4	.15
	F=3.38*	F=1.77	F=.516	

*Significant at the 0.05 level.

Serum cortisol. Problems in sampling restricted the serum cortisol determinations to experimental periods four and five. It was impossible to draw enough blood from smaller fish to yield the 1 ml of serum necessary for analysis; therefore, many samples were pooled. Cortisol levels ranged from 1.04 to 5.52 µg/100 ml serum (Appendix, Table 10).

Blood pH. When blood has been exposed to air, CO_2 rapidly dissipates, causing an increase in blood pH. In order to compare small differences which might be significant, blood pH should be measured anaerobically to prevent changes due to loss of CO_2 (Wedemeyer, pers. comm.). Apparatus for anaerobic measurement of blood pH was not available; therefore, pH was limited to aerobic measurement during the study. Consequently, only gross changes (>0.5 pH units) in blood pH could be recognized.

Measurements of blood pH were limited to experimental periods one, four and five. No gross changes were observed.

Disease observations

A. salmonicida was isolated from 41 percent of the 106 Furunculosis Agar slants. Bacterial growth other than A. salmonicida occurred in 39 percent of the samples, and 20 percent yielded no growth (Table 7).

Many of the fish collected from the trap exhibited external lesions and ulcers. During experimental periods four and five, a number of fish were observed to have localized areas of unidentified fungus, commonly associated with previous mechanical injury.

Table 7. Reaction of kidney smears on Furunculosis Agar slants, Temple Fork study, 1972.

Period	Number of Slants	Positive	Negative with Unidentified Bacterial Growth	Negative No Growth
1	1	1	0	0
2	15	9	3	3
3	33	14	11	8
4	32	14	11	7
5	25	6	16	3
Total	106	44	41	21
% of Total		41.5	38.7	19.8

A total of 12.8 percent of the fish recovered during experimental periods one and two had lost their tags. Associated with the tag loss were large open wounds, apparently caused by secondary infection, at the point of tag insertion. Realizing this problem, fish tagged for experimental periods three, four and five were treated differently. The tagging needle was dipped in alcohol and distilled water between each tag insertion, and as a result, loss of tags and signs of infection were significantly lower for these periods (Table 8).

Post-experiment Population

The post-experiment survey of the study area yielded an estimated population of 596 (S.E. = 16). Subsequent sampling below the trap site confirmed that fish not found in the study area had moved downstream. Although stream residence varied from 2 to 14 weeks, no

significant change ($\alpha = 0.05$) in length or weight of captured fish from time of stocking was observed.

Table 8. Number of fish exhibiting tag loss and percent of total captured, Temple Fork study, 1972.

Period	Number Captured	Number Without Tag	Percent of Total
1	90	11	12.40
2	128	17	13.30
3	62	5	8.10
4	100	3	3.00
5	130	2	0.01

DISCUSSION

Mortality in Stocked Trout

Influence of stream flow and temperature

Stream conditions which favor survival of planted catchables gradually improved throughout the summer. Also, the average size of planted fish increased during this time. If stressful conditions existed at any time during the study, it would be expected that during experimental period one, evidence of stress would be most pronounced. Although determinations of interrenal ascorbic acid and serum cortisol were not made during the first period, the trend of decreased mortality throughout the summer would tend to support this contention.

Mortalities of stocked trout varied among experimental periods. Without precise estimates of density of stocked trout at any one time, however, it was impossible to segregate the influence of physical stream parameters and density of fish upon total mortality. Since the majority of all observed mortality occurred during captivity in live boxes, it would be erroneous to correlate observed mortalities to physical stream characters.

Physiological response to stress

Interrenal ascorbate determinations indicated that handling and transportation did not stress the hatchery rainbow trout enough to cause vitamin C depletion. Similar results from fish captured in the trap after planting indicated that the short residence in the lotic environment did not stress the fish. Since interrenal ascorbic acid

depletion has been used to indicate activation of the pituitary-interrenal axis (Wedemeyer, 1972), it appears that adrenocorticotrophin production did not occur in the study fish.

The limited determinations of serum cortisol support this contention. Although results of the two physiological parameters from individual fish were not available for comparison, due to pooling of blood samples, all cortisol determinations fell within the expected normal range (Wedemeyer and Chatterton, 1970).

Extreme variation was found in results of vitamin C determinations (range 11.3 - 387.8 µg/g kidney). Wedemeyer and Chatterton (1970) have reported a normal range of interrenal ascorbic acid for yearling hatchery rainbow trout between 102 - 214 µg/g kidney. Since kidneys were weighed in the field using a pan balance, extremes in the results of this study may have been due to inaccurate weighing of kidney tissue. This is a critical step in the analysis, since the weight of kidney determines the amount of metaphosphoric acid used for dilution. A low estimation of kidney weight would yield a more concentrated sample after dilution resulting in high levels of ascorbic acid. The reverse would be true for high estimation of kidney weight. One possible solution to this problem would be to freeze the whole kidney after extraction from the fish and then the sample could later be weighed on an analytic balance in the laboratory prior to vitamin C analysis.

Although interrenal vitamin C depletion was not evident among groups, some individuals did exhibit low levels. Of the moribund fish examined, many had subnormal levels of ascorbic acid indicating that stress may have been involved with the observed mortality. Serum

cortisol determinations were not made for moribund fish, so comparisons with ascorbate were not possible.

Disease observations

The occurrence of furunculosis in rainbow trout stocked during this study revealed disease to be a major factor in the observed mortalities. A. salmoniciola was isolated on approximately 41 percent of the 106 Furuncalosis Agar slants, and 40 percent exhibited some other bacterial growth. Although no attempt was made to identify these bacteria, it is possible that they may have been causitive agents of other diseases.

McCraw (1952) reported that Furunculosis is a stress mediated disease. This fact should be of particular interest to the hatchery program. Although the results of this study show that significant depletion of ascorbic acid and increases in serum cortisol did not occur, the possibility of stress in these fish should not be eliminated. Wedemeyer (1972) found that handling alone stressed yearling rainbow trout enough to produce osmoregulatory and metabolic dysfunctions, but pituitary activation, as measured by interrenal ascorbic acid, did not occur. It is possible that the levels of stress encountered in the present study, although not great enough to stimulate production of adrenocorticotrophin, could be sufficient to lower the resistance of fish to disease.

Observations in other waters on the fate of stocked trout taken from infected hatchery stockes are lacking. Disease has not been discussed in the literature as a possible cause for delayed mortality. Results from this study indicate that this problem should be examined in detail.

CONCLUSIONS AND RECOMMENDATIONS

It appears that in Temple Fork of the Logan River levels of stress of stocked catchable-sized rainbow trout were not extensive enough to produce interrenal ascorbic acid depletion or increased levels of serum cortisol. Instead, most mortalities were attributed to disease carried from the hatchery. Apparently, handling, transportation and planting did induce low stress levels sufficient to mediate diseases such as Furunculosis.

The need for further research is indicated. Future studies should involve a more efficient method of monitoring downstream fish movement so that a more accurate estimation of mortality can be made. The use of additional sensitive measurements as indicies of physiological status, such as plasma glucose, calcium and cholesterol, would enable a more accurate estimation of the level of stress. Refinement of sampling technique for interrenal ascorbic acid, especially weighing of kidney samples, should lower the variability considerably. Extensive laboratory experimentation to better define the effect of different levels of stress on fish would be beneficial in evaluating levels encountered in the field. Finally, more extensive field sampling for infectious diseases known to occur in hatchery stocks would aid in estimating the effect of planting fish from such stocks.

LITERATURE CITED

Adelman, H. M., J. L. Bingham, and J. L. Maatch. 1955. The effect of starvation upon brook trout of three sizes. Prog. Fish-Cult. 17(3):110-112.

Anonymous. 1956. Prog. Fish-Cult. 18(1):14.

Black, E. C. 1955. Blood levels of hemoglobin and lactic acid in some freshwater fishes following exercise. J. Fish Res. Bd. Canada 12(6):917-929.

Black, E. C. 1956. Appearance of lactic acid in blood of kamloops and lake trout following live transportation. Canadian Fish Cult. No. 18, pp. 20-27.

Black, E. C. 1957. Alterations in the blood level of lactic acid in certain salmonid fishes following muscular activity I. kamloops trout, Salmo gairdneri. J. Fish. Res. Bd. Canada 14(2):117-134.

Black, E. C. 1957. Hyperactivity as a lethal factor in fish. J. Fish. Res. Bd. Canada 15(4):573-586.

Brett, J. R. 1958. Implications and assessment of environmental stress. Invest. Fish-Power Prob., U. Brit. Col., pp. 69-83, (Edited by P. A. Larkin).

Bricker, M. J. 1970. Effects of starvation and time of stocking on survival of stocked rainbow trout, Salmo gairdneri. M.S. Thesis, Utah State University, 71 pp.

Calhoun, A. 1966. Habitat considerations for catchable trout. Proc. Western Assoc. State Game and Fish Comm. 46:254-256.

Clark, B. R., and R. T. Rubin. 1969. New fluorometric method for the determination of cortisol in serum. Anal. Biochem. 29:31-39.

Dell, M. B. 1968. A new fish tag and rapid cartridge-fed applicator. Trans. Amer. Fish. Soc. 97(1):57-59.

Dickson, I. W., and R. H. Kramer. 1971. Factors influencing scope for activity and active and standard metabolism of rainbow trout (Salmo gairdneri). J. Fish. Res. Bd. Canada 28:587-596.

Dwyer, W. P., and R. H. Kramer. 1973. The influence of temperature on scope for activity in cutthroat trout, Salmo clarki. J. Fish. Res. Bd. Canada (In Press).

Fagerlund, U. H. M. 1967. Plasma cortisol concentration in relation to stress in adult sockeye salmon during the freshwater stage of their life cycle. Gen. Comp. Endocr. 8:197-207.

Fry, F. E. J. 1947. Effects of the environment on animal activity. Univ. Tor. Stud., Biol. Ser. No. 55. Pub. Ont. Fish. Res. Lab., No. 68, 62 pp.

Griffin, P., S. F. Snieszko, and S. Friddle. 1953. A new adjuvant in the diagnosis of fish furunculosis caused by Bacterium salmonicida. Vet. Med. 48:280-282.

Hane, S., O. H. Robertson, B. C. Wexler, and M. A. Krupp. 1966. Adrenocortical response to stress and ACTH in Pacific salmon (Oncorhynchus tshawytscha) and steelhead trout (Salmo gairdneri) at successive stages in the sexual cycle. Endocrinology 78:791-800.

Hochachka, P. W., and A. C. Sinclair. 1962. Glycogen stores in trout tissues before and after stream planting. J. Fish. Res. Bd. Canada 19(1):127-136.

Horton, H. F. 1956. An evaluation of some physical and mechanical factors important in reducing delayed mortality of hatchery-reared rainbow trout. Prog. Fish-Cult. 18(1):3-14.

Kramer, R. H. 1969. Approaches to develop fish-suitability criteria. Proc. Western Assoc. State Game and Fish Comm. 49:225-233.

Little, R. G. 1966. Are catchable trout a substitute for good habitat. Proc. Western Assoc. State Game and Fish Comm. 46:271-272.

Locke, F. E. 1966. The use of catchables in relation to habitat. Proc. Western Assoc. State Game and Fish Comm. 46:250-253.

McCraw, B. M. 1952. Furunculosis of fish. Special Scientific Report, Fisheries No. 84, 87 pp.

Meigs, R. C., and C. Mullenbach. 1955. Utilization of hatchery fish. Prog. Fish-Cult. 17(2):87-89.

Miller, R. B. 1951. Survival of hatchery-reared cutthroat trout in an Alberta stream. Trans. Amer. Fish. Soc. 81:35-42.

Miller, R. B. 1953. Comparative survival of wild and hatchery-reared cutthroat trout in a stream. Trans. Amer. Fish. Soc. 83:120-130.

Miller, R. B. 1955. Trout management research in Alberta. 20th N. Amer. Wildl. Conf. pp. 242-252.

Miller, R. B. 1958. The role of competition in the mortality of hatchery trout. J. Fish. Res. Bd. Canada 15(1):27-45.

Miller, R. B., A. C. Sinclair, and P. W. Hochachka. 1959. Diet, glycogen reserves, and resistance to fatigue in hatchery trout. J. Fish. Res. Bd. Canada 16(3):321-327.

Needham, P. R. 1959. New horizons in stocking hatchery trout. Trans. 24th N. Amer. Wildl. Conf. pp. 395-407.

Pearson, W. D. 1970. Drift of Oligophlebodes sigma and Baetis bicaudatus in a mountain stream. Ph.D. Dissertation, Utah State University. 101 pp.

Pearson, W. D., and R. H. Kramer. 1972. Drift and production of two aquatic insects in a mountain stream. Ecol. Mon. 24:365-385.

Polk, A., T. L. Flanagan, and E. J. Van Loon. 1960. Rapid method for determination of ascorbic acid in urine. Clin. Chem. 6:558-563.

Reimers, N. 1957. Some aspects of the relation between stream foods and trout survival. Cal. Fish and Game 43(1):43-69.

Seaman, W. R. 1966. Use of catchable trout in Colorado. Proc. Western Assoc. State Game and Fish Comm. 46:248-250.

Schoettger, R. A., and E. W. Steucke, Jr. 1970. Synergic mixtures of MS-222 and quinaldine as anesthetics for rainbow trout and northern pike. Prog. Fish-Cult. 32(4):202-205.

Schuck, H. A. 1948. Survival of hatchery trout in streams and possible methods of improving the quality of hatchery trout. Prog. Fish-Cult. 10(1):3-14.

Stobo, W. T. 1972. The effects of dart tags on yellow perch. Trans. Amer. Fish. Soc. 101(2):365-366.

Stroud, R. H. 1970. Future of fisheries management in North America. pp. 291-308. In Norman Benson (Ed.) A Century of Fish Culture. Special Publication No. 7, American Fisheries Society, Washington, D.C.

Twedt, T. M. 1973. Trout movement in a small mountain stream. M.S. Thesis, Utah State University, 32 pp.

U.S. Dept. of the Interior. 1962. Sport fishing - today and tomorrow. A report to the O.R.R.R.C. by the Bureau of Sport Fisheries and Wildlife. U.S. Govt. Printing Office, Washington, D.C.

Wedemeyer, G. 1969a. Stress-induced ascorbic acid depletion and cortisol production in two salmonid fishes. Comp. Biochem. Physiol. 29:1247-1251.

Wedemeyer, G. 1969b. Pituitary activation by bacterial endotoxins in rainbow trout (Salmo gairdneri). J. Bact. 100(1):542-543.

Wedemeyer, G. 1970a. Stress in disease resistance. In S. F. Snieszko (Ed.) A Symposium on Diseases of Fishes and Shellfishes. Special Publication No. 5, American Fisheries Society, Washington, D.C.

Wedemeyer, G. 1970b. Stress of anesthesia with MS-222 and benzocaine in rainbow trout (Salmo gairdneri). J. Fish. Res. Bd. Canada 27(5):909-914.

Wedemeyer, G. 1972. Some physiological consequences of handling stress in juvenile coho salmon (Oncorhychus kisutch) and steelhead trout (Salmo gairdneri). J. Fish. Res. Bd. Canada 29(12):1780-1783.

Wedemeyer, G., and K. Chatterton. 1970. Some blood chemistry values for the rainbow trout (Salmo gairdneri). J. Fish. Res. Bd. Canada 27(6):1162-1164.

Wedemeyer, G., and K. Chatterton. 1971. Some blood chemistry values for the juvenile coho salmon (Oncorhynchus kisutch). J. Fish. Res. Bd. Canada 28(4):606-608.

Wedemeyer, G., A. J. Ross, and Lynwood Smith. 1968. Some metabolic effects of bacterial endotoxins in salmonid fishes. J. Fish. Res. Bd. Canada 26(1):115-122.

Wood, E. M. 1953. A century of fish culture, 1853-1953. Prog. Fish-Cult. 15(4):147-162.

Zippin, C. 1958. The removal method of population estimation. J. Wildl. Man. 22(1):82-90.

APPENDIX

Table 9. Range of body weight (g) and interrenal ascorbic acid levels (µg/g kidney) of fish sampled for each treatment during successive experimental periods, Temple Fork study, 1972.

Period	Treatment	Weight	Level of Ascorbate
	Control	125-234	97.9-308.0
2	Transported	99-206	107.3-257.4
	Planted	73-229	52.3-376.8
	Control	115-210	81.4-264.0
3	Transported	99-272	24.0-264.0
	Planted	100-219	113.8-319.0
	Control	143-211	167.3-275.0
4	Transported	119-219	77.0-345.4
	Planted	90-253	70.4-298.1
	Control	110-327	111.1-227.7
5	Transported	89-312	46.2-350.9
	Planted	152-311	11.0-374.0

Table 10. Serum cortisol levels (µg/100 ml serum) for control, transported and planted fish pooled over experimental periods, Temple Fork study, 1972.

Control	Transported	Planted
1.04	2.38	2.54
3.71		2.54
1.23		5.22
		3.73
		3.73
		1.34
		3.43
		5.52
		2.76
		4.09

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