

EFFECTS OF ELECTROFISHING ON LONG-TERM GROWTH  
AND MORTALITY OF WILD RAINBOW TROUT

by  
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A thesis submitted in partial fulfillment  
of the requirements for the degree  
of  
Master of Science  
in  
Fish and Wildlife Management

MONTANA STATE UNIVERSITY  
Bozeman, Montana

May 1994

APPROVAL

of a thesis submitted by

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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## ACKNOWLEDGEMENT

I wish to express my sincere appreciation to the following people for their support and encouragement throughout this study. Wade Fredenberg brought much needed attention to an important issue. Drs. Robert White and Harold Picton reviewed the manuscript. Dee Topp was a great secretary. Thanks to all the folks at the U.S. Fish Technology Center, especially Pat Dwyer, for assistance and the use of the facility. The Montana Department of Fish, Wildlife and Parks provided funding and field assistance. A special thanks to all my fish friends at R-5 who got me started in this business. Thanks to my fellow graduate students, especially my office mates for their help in the field, enduring the odor of dead fish and making sure I got an occasional "wake up call".

I would like to extend a special thanks to my major professor, Dr. Thomas McMahon, for being a great teacher, providing critical insight, direction and support in all phases of this study and for enduring my short attention span during hunting season. Finally, I owe special recognition to my wife Sue, my best friend, field assistant, editor and Mom.

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## ABSTRACT

Spinal injury rates as high as 67% have been reported in large (>300 mm long) rainbow trout (*Oncorhynchus mykiss*) collected with pulsed direct current. This study was designed to evaluate how incidence and severity of electrofishing-induced spinal injury in wild rainbow trout affects long term growth and mortality. Three test groups of 241, 309, and 316 fish were collected from the Gallatin River using three waveforms: smooth (SM), 60-Hz half pulse (HP), and 60-Hz full pulse direct current (FP). Spinal injury rates were highest in fish collected with FP (54 %), followed by HP (40 %) and SM (12 %), while class 3 spinal injury (fracture of one or more vertebrae) levels were similar in all three test groups (6% SM, 6% HP, 10% FP). Fish from the three test groups were placed into a 0.61 hectare pond for approximately 1 year where they were subsampled after 100 days and all remaining fish removed after 1 year. Growth and mortality were compared by test group and by severity of electrofishing-induced spinal injury. There was no significant difference in short-term (100 days) or long-term (1 year) growth between the three test groups. However, spinally injured fish exhibited lower growth, less weight gain, and lower condition factor increase than uninjured fish. Test group and severity of spinal injury did not appear to influence overall survival at the termination of the study (54 % SM, 58 % HP and 60 % FP). Although specimens collected with FP and HP exhibited higher total spinal injury rates than those collected with SM, the long-term impacts of this injury were not manifested in decreased growth and survival.

## INTRODUCTION

Electrofishing has been proven to be directly responsible for spinal and soft tissue injuries in fish. Injuries are thought to occur when fish intercept the electric field which elicits powerful contractions of the body musculature. These contractions are believed to occur simultaneously on both sides of the body, thus generating opposing forces which can compress, misalign or fracture vertebrae (Cowx and Lamarque 1990). Sharber and Carothers (1988) determined that spinal injury was found in 44% to 67% of large (>300 mm long) rainbow trout (*Oncorhynchus mykiss*) electrofished with pulsed direct current (PDC). That study provided the impetus for further investigations dealing with the magnitude and impacts of electrofishing-induced injury on growth and mortality in several freshwater fishes. The Alaska Department of Fish and Game (ADFG) conducted a study on the Kenai River which provided estimates of short-term mortality and injury for large rainbow trout exposed to electrofishing with PDC (Holmes et al. 1990). Forty-one percent spinal injury rate and 14% short-term (96 hours) mortality was observed, thus prompting ADFG to place a moratorium on electrofishing in all waters in Alaska containing trophy rainbow trout (Reynolds undated). A study by the Montana Department of Fish, Wildlife and Parks (MDFWP) on the Missouri River in 1988 to assess electrofishing-induced injury revealed 50 - 70 % spinal injury rates in large rainbow trout (Fredenberg 1992). In his follow-up study involving trout collected from across the state, Fredenberg (1992) found that among 693 electrofished trout, there were 769 hemorrhages and 2,647 injured vertebrae. Present information suggests that electrofishing injury of rainbow trout depends on waveform and pulse shape, rate, duration, and intensity (Gatz et al. 1986).

High injury rates of fish collected with several different PDC waveforms led the MDFWP to issue electrofishing guidelines recommending the use of pulse frequencies of 20 pulses per second or less, short pulse duration (5 msec.), and the lowest possible voltages to minimize injury (Fredenberg 1992). Several studies have observed increased injury rates as a function of the pulse shape and frequency (Spencer 1967; Sharber and Carothers 1988; Cowx and Lamarque 1990; Taube 1992). Fredenberg (1992) observed that increasing pulse frequencies increased incidence of spinal injury and hemorrhage. The lowest injury rate was with smooth DC, followed by half pulse DC (intermediate injury), and full pulse DC (highest overall rates). The use of direct current (as opposed to AC or PDC) is recommended to reduce incidence of injury by Reynolds (undated). In his review of electrofishing, Snyder (1993) also states that when direct current is used, it should be as smooth as possible to minimize the risk of spinal injury.

The long-term effects of electrofishing injury on the growth and survival of wild rainbow trout populations remain largely unknown (Reynolds undated). While several studies have assessed the effects of electrofishing on growth and survival of hatchery rainbow trout and other species (Spencer 1967; Hudy 1985; Gatz et al. 1986; Gatz and Adams 1987; Roach 1992; Taube 1992), investigations to date have not examined the fate of injured wild fish over the long-term (1 year or greater). Furthermore, the potential healing of electrofishing-induced spinal injuries has yet to be investigated.

Based on the need for better information on the long-term effects of electrofishing-induced injury on fishes, this study was designed to:

- (1) determine the short-term and long-term growth and survival of wild rainbow trout electroshocked with three different DC waveforms;
- (2) compare the relative growth and survival rates of spinally injured and non-injured electroshocked fish; and
- (3) evaluate healing of electrofishing-induced spinal injuries.

## STUDY AREA

The growth and survival portion of this study was conducted in an irrigation storage pond located on the campus of Montana State University (MSU). This manmade pond is 0.61 hectare with a maximum depth of 4.5 m, a mean depth of 3.5 m, a storage capacity of approximately 19,000,000 L and a regular bottom composed of mud and gravel. The pond is fed by diverted Hyalite Creek water. No surface outflow exists as water is pumped for campus irrigation. The inlet was screened to prohibit any fish movement out of the pond. Water levels fluctuated slightly during summer irrigation months but remained constant throughout the rest of the year. The pond perimeter is secured with a 3 m barbed wire and chain link fence to discourage access.

Prior to introduction of test fish into the pond, existing fish were removed with gill nets. Sixteen brook trout (average length 40.1 cm, average weight of 1.1 kg) were captured the first night, but none the following two nights. These fish were likely transients from Hyalite Creek that were diverted with irrigation water and became trapped in the pond when the ditches were shut down. Freshwater shrimp (*Gammarus lacustris*) were abundant in the littoral areas of the pond. Plankton tows were run on August 10, 1992 yielding abundant Daphnia sp. and Diaptomus sp.. Dissolved oxygen and water temperature measurements recorded during winter months ranged from 8-11.5 ppm and 1.2-5 °C with no apparent stratification. Summer temperatures ranged from 10 to 26 °C with the peak temperature recorded on August 1, 1992.

## METHODS

### Fish Collection

I tested the hypothesis that there is no difference in growth and survival of fish collected with three different DC waveforms by collecting 1,036 wild rainbow trout (153 - 388 mm fork length) via single pass, mobile electrofishing (Fredenberg 1992). Conventional wattage settings on the shocker box were selected as these typically produce high fish collecting efficiency for rivers in the region (Wayne Black, MDFWP, pers. comm.) (Table 1). Fish were collected from a 14 km reach of the Gallatin River, Montana on July 27 and 28, 1992. The sequence of waveforms was smooth (SM), half pulse (HP) and full pulse (FP) (Figure 1). Shocking with each waveform used continued downstream until the target number of fish for each group (330) was captured. This reach of the Gallatin was selected due to a high density of rainbow trout and it had not been electrofished for several years, thus eliminating the possibility of collecting fish that may have electrofishing-induced injury from previous electrofishing operations.

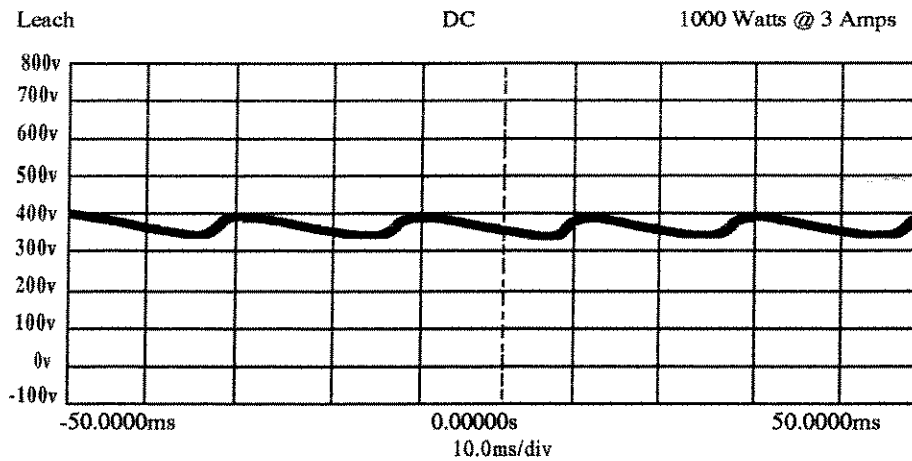
The type of equipment, electrode array design, electrofishing setting, water temperatures, and conductivities associated with test fish collection are summarized in Table 1. Peak voltages were measured at seven measured intervals (Figure 2) from the anode using a set of metal contacts 1 cm apart mounted on a probe and connected to an oscilloscope. These measurements were recorded in order to differentiate power gradients (v/cm) of the three waveforms at specified distances from the anode where the majority of fish netting within the electrical fields occurs. Documentation of electrical and physical variables in this study was conducted to further standardize the reporting of these variables in electrofishing experiments as recommended by Reynolds (undated).

Following collection, fish were transported by hatchery truck to raceways at the U.S. Fish and Wildlife Service Fish Technology Center in Bozeman, Montana. Transport water was tempered for approximately 30 minutes prior to fish being released into outdoor raceways where test groups were held separately.

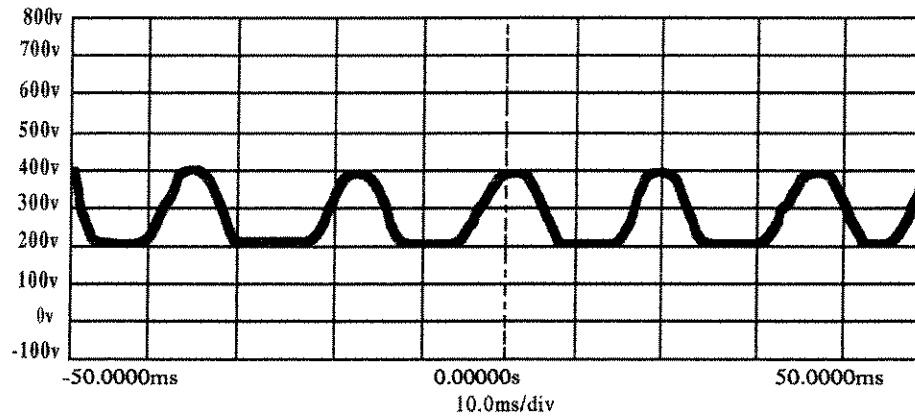
Table 1. Physical parameters and equipment specifications associated with the collection of wild rainbow trout from the Gallatin River on July 27 - 28, 1992.

<b>Water Temperature (°C)</b>	13 -16
<b>Conductivity (umhos/cm)</b>	260
<b>Water Clarity</b>	Approximately 1 m visibility
<b>Boat</b>	3.6 m Fiberglass Drift
<b>Generator</b>	3,000 Watt Gillette
<b>Anode Configuration</b>	25.4 cm Aluminum Triangle (2.54 cm)
<b>Cathode Configuration</b>	1.2 m <sup>2</sup> Stainless Steel Plate
<b>Shocker Box Type</b>	Leach Box, 220 Volt
<b>Shocker Setting</b>	1,000-1,500 Watt Output

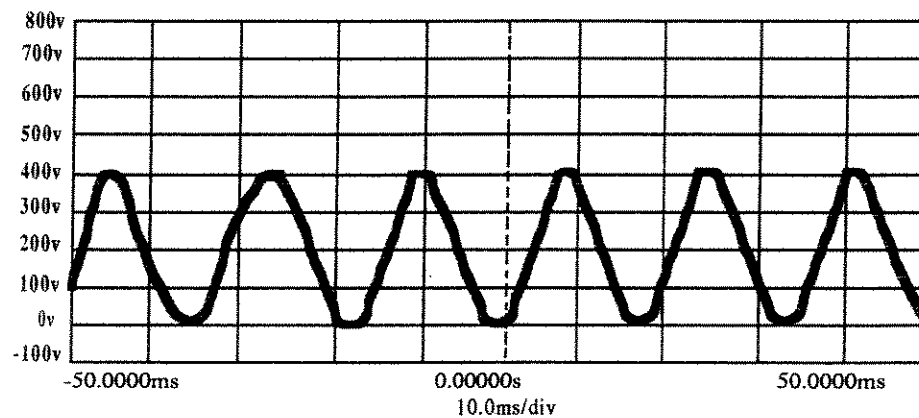




A.) Smooth Direct Current (SM)



B.) Rectified AC - 60Hz Sine Half Pulse Direct Current (HP)



C.) Rectified AC - 60Hz Sine Full Pulse Direct Current (FP)

Figure 1. Electrical waveforms used to collect the three test groups of wild rainbow trout from the Gallatin River, Montana on July 27 and 28, 1992. Waveform diagrams generated using a digitizing oscilloscope at 1000 watts and 3 amps (Fredenberg 1992).

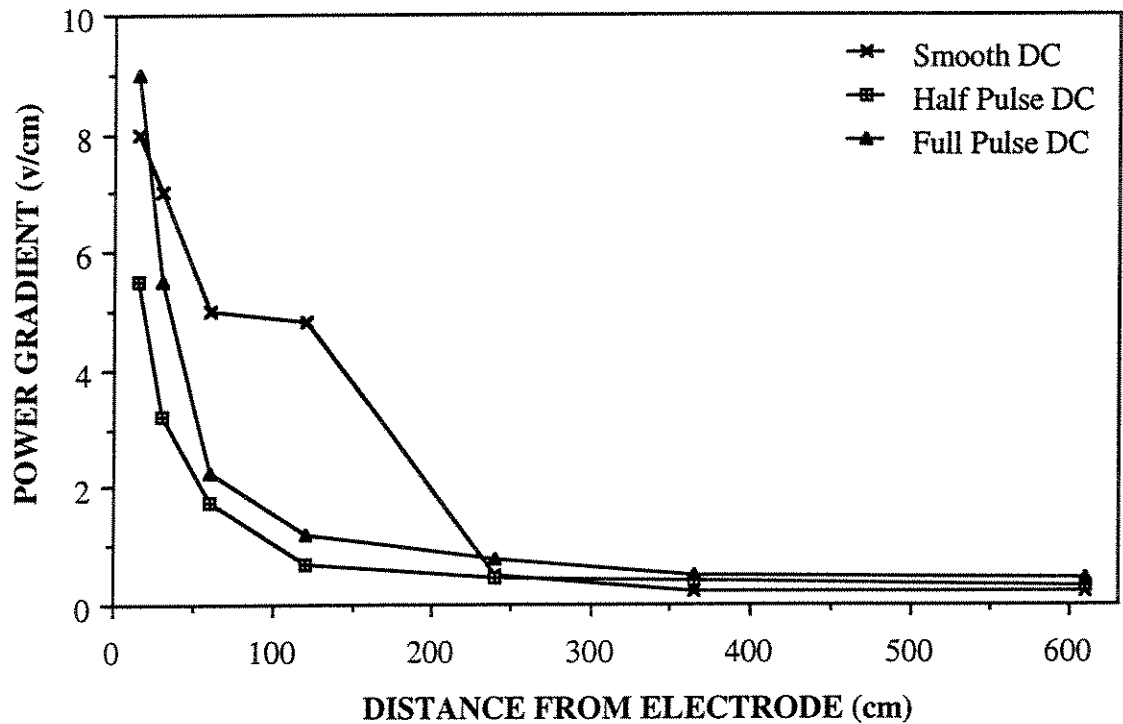


Figure 2. Peak voltage gradients for the three waveforms used to collect wild rainbow trout from Gallatin River on July 27 and 28, 1992.

#### Weighing, Tagging and X-raying

After 24 h, trout within each test group were moved to indoor raceways for x-raying and tagging. Fish were anesthetized with MS-222 (3.5-4.5 ml of 5% tricaine methanesulfonate per gallon of water) in groups of ten, tagged with a coded wire tag in one of three unique locations to identify test groups, and marked with a visible implant (VI) tag in the postorbital adipose tissue of each fish to identify individuals (Figure 3). Weights and fork lengths were measured and each fish was then placed in a second tub containing 5.0 ml MS-222 per gallon of water where they were held until the entire group of ten fish had been processed. VI tags were verified and fish were x-rayed (right lateral view) with a portable X-ray machine (Minxray X750G) using standard veterinary x-ray film (PC Konica

Blue, 35.5 X 43 cm). Exposure varied from 0.6 - 0.8 s depending on fish size. Power setting and source to image distance remained constant at 50 kilovolts and 760 mm, respectively. Immediately following x-ray, fish were placed in oxygenated holding tanks on the hatchery truck. Upon completion of processing of a test group, fish were transported to the MSU pond. Water in the hatchery truck (12°C) was gradually tempered over an hour to that of the pond (24°C) before releasing fish. A total of 866 fish were released into the MSU pond on July 29-30, 1992 (Table 2). Sample sizes for the three test groups released into the pond were not equal due to the escape of some SM test group fish from the outdoor raceway. Nineteen HP and 63 FP fish were randomly sacrificed prior to introduction of test fish into the pond to keep sample sizes of the test groups similar. Initial length frequencies of the three test groups were not significantly different (SM = 25.3 cm  $\pm$  0.29, HP = 24.4 cm  $\pm$  0.24, FP = 24.5 cm  $\pm$  0.22) ( $H = 3.44$ ,  $P = 0.18$ ).

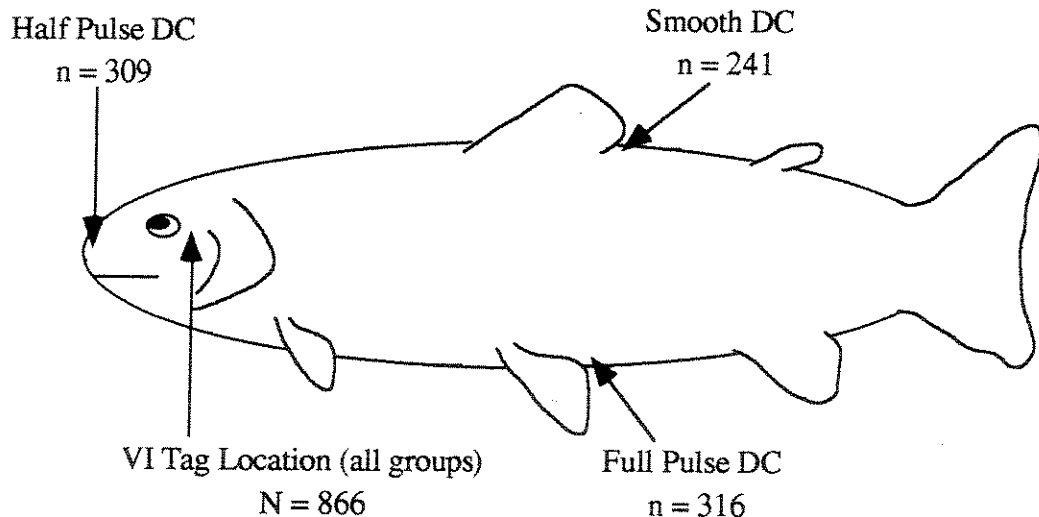


Figure 3. Coded wire and VI tag locations and the sample sizes of each test group of fish that were introduced into the MSU campus pond.

Table 2. Number of wild rainbow trout collected with the three waveforms from the Gallatin River and dates of release into the MSU pond.

	DATE	SM	HP	FP
# COLLECTED	7/27/92	325	294	—
# COLLECTED	7/28/92	—	34	379
# RELEASED	7/29/92	158	284	—
# RELEASED	7/30/92	83	25	316
TOTAL IN POND	7/30/92	241	309	316

### Survival and Growth

The pond was monitored for mortalities twice daily (0900 and 1800) by walking the pond perimeter. All mortalities collected were frozen for later analysis. After the number of mortalities had declined to near zero (Nov., 1992), the pond was monitored three to five times per week.

To document short-term growth rates, a sample of 13-14% of each test group was removed from the pond approximately 100 d post-release (7 Nov 1992) using box traps and short-term gill net sets. All fish collected were sacrificed with an overdose of MS-222 followed by the collection of weight and length data, test group identification, and x-raying of those fish containing a VI tag. Several of the more severely injured fish were necropsied to determine the extent of healing.

The growth and survival study was terminated on July 1, 1993 by introducing rotenone (5% formulation) at a concentration of 1mg/L into the pond. Due to the lack of aquatic vegetation, water visibilities of approximately 1.5 m, and favorable water quality criteria, it was determined that rotenone treatment would be the most efficient means of total

fish removal. Fish were collected from the pond for seven days post-rotenone treatment after which they were weighed, measured, and x-rayed if the VI tag was still present.

Trout age classes were assigned using age data from 425 scale samples collected from wild rainbow trout from the lower Gallatin River in September 1984 and 1989 by MDFWP (Mark Lere, MDFWP, pers. comm.). These two age class estimates were averaged to estimate the age of rainbow trout used in this experiment. Six age classes of fish were collected with age class I individuals averaging 14.5 cm, age class II at 18.9 cm, age class III at 24 cm, age class IV at 28.7 cm, age class V at 32.8 cm and age class VI at 35.7 cm.

Growth of rainbow trout in the pond was compared with that of rainbow trout in more natural conditions of the Gallatin River. Growth data from the Gallatin River were obtained from a 1983 - 84 population survey conducted by the MDFWP. This estimate was collected on the 3.5 km Jack Smith Bridge section of the Gallatin River (Mark Lere, MDFWP, pers. comm.).

#### Incidence and Healing of Spinal Injury

Spinal injury to fish after electrofishing was quantified with x-rays. Severity of spinal injury was rated by the standardized evaluation ranking criteria that was proposed by Reynolds (undated) (Table 3).

X-rays were developed, shuffled and randomly selected to eliminate any bias in rating the spinal injuries due to prior knowledge of the general injury rates of a particular waveform. Naturally occurring spinal abnormalities, identified by calcification and fusing of vertebrae (Sharber and Carothers 1988; Fredenberg 1992), were not ranked or included in the analysis. For each fish, the maximum spinal injury rating of any single vertebrae was designated the maximum injury rating. Evaluation of electrofishing-induced

hemorrhage was conducted on a small group of FP and HP fish as per the protocol established by Fredenberg (1992). These fish were sacrificed with an overdose of MS-222 and frozen until necropsies were conducted.

Incidence of spinal injury was the percentage of fish collected with each waveform that exhibited any level of spinal injury. Severity of injury was calculated as a percentage of each of four injury classes.

Healing of spinal injuries was evaluated at the end of the study for 38 fish exhibiting spinal injuries. These fish were randomly chosen and x-rayed and rated using the same criteria that was used at the start of the study. X-rays were first rated without knowledge of initial injuries; then degree of healing was determined by comparing individual vertebrae between the two x-rays.

Table 3. Spinal and soft tissue injury evaluation ranking criteria used to evaluate spinal damage as determined from x-rays and necropsies (after Reynolds undated).

X-RAY		CRITERIA	
0		No spinal damage apparent.	
1		Compression (distortion) of vertebrae only.	
2		Misalignment of vertebrae, including compression.	
3		Fracture of one or more vertebrae or complete separation of two or more vertebrae.	
HEMORRHAGE		NECROPSY CRITERIA	
0		No hemorrhage apparent.	
1		Mild hemorrhage; one or more wounds in the muscle, separate from the spine.	
2		Moderate hemorrhage; one or more small ( $\leq$ width of two vertebrae) spinal wounds.	
3		Severe hemorrhage; one or more large ( $\geq$ width of two vertebrae) spinal wounds.	

### Data Analysis

The incidence and severity of spinal injury was compared by test group (waveform treatment) and by age class using the log-likelihood ratio (G-statistic; Zar 1984). Growth and survival of wild rainbow trout was compared by test group and by severity of spinal injury. Growth was analyzed as the change in length, weight and condition ( $K=W/L^3 \cdot 10^5$ ) over short-term (100 d) and long-term (1 yr) intervals. Only data from fish that retained the VI tag were used in the analysis; poor retention of VI tags (50 %) reduced sample sizes. Differences in growth and condition by test group and injury class were analyzed by Kruskal-Wallis nonparametric analysis of variance and by a multiple comparison test for unequal sample sizes (Zar 1984). Long-term survival was determined as the original number released minus the number of fish in each test group remaining in the pond at the end of the study. Fish collected as mortalities and the sample removed for the short-term growth analysis were subtracted from the original number released.

All statistical testing was considered significant at the  $P \leq 0.05$  level. STATVIEW SE + GRAPHICS® (1991) and STATGRAPHICS® (1989) statistical packages were used to perform all statistical calculations.

## RESULTS

### Injury Rates

Spinal injury rating was achieved through the systematic evaluation of x-rays, based on established criteria (Table 3), for the 866 fish used in this study. The evaluation process was very precise as the criteria were found to be unambiguous (S. Dalbey, pers. obser.). Less than one percent of the 866 fish x-rayed were difficult to read due to fish being too small (small vertebrae) or due to obscured x-rays from fish movement during x-ray photography.

Initial spinal injury rates differed significantly between the three test groups in both incidence ( $G = 115.71$ , 2 d.f.,  $P = 0.0001$ ) and severity ( $G = 49.85$ , 6 d.f.,  $P = 0.0001$ ). The SM test group had the lowest incidence of injury (12%); of these, 2% were class "1" spinal injury, 4% class "2", and 6% class "3". The HP test group had 40% injury rate, with severity of 10% class "1", 24% class "2", and 6% class "3". The FP group exhibited the highest rate of spinal injury (54%); of these 14% were class "1", 30% class "2" and 10% class "3" (Figure 4). There was no significant difference in the incidence of class "3" injury among the three test groups, but there were significant differences in class "1" and "2" among the three test groups (Figure 4;  $G = 45.06$ , 5 d.f.,  $P = 0.0001$ ).

The overall incidence of spinal injury did not vary significantly between age classes when fish from all test groups were combined (Figure 5;  $G = 8.389$ , 5 d.f.,  $P = 0.1489$ ). Age class I fish exhibited the lowest injury rate (25%) while age III fish had the highest



(42%). There were only two individuals collected in age class VI and both of these fish were uninjured (Figure 5).

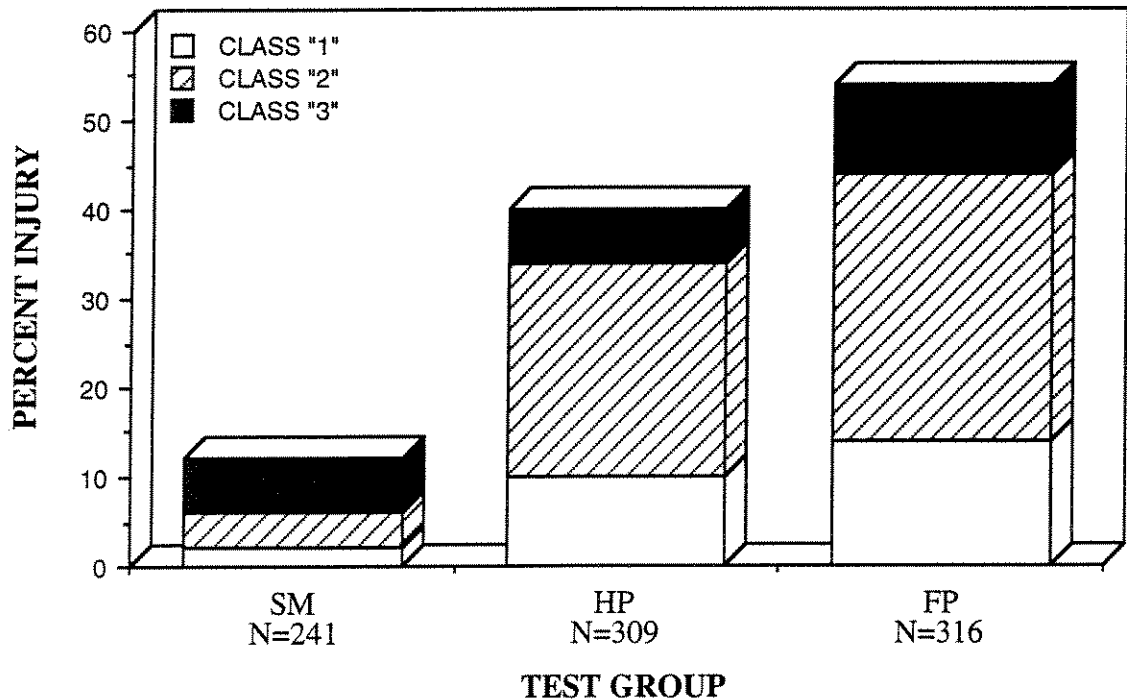


Figure 4. Initial percentages of three spinal injury classes exhibited by fish collected using three different direct current waveforms from the Gallatin River, Montana.

Severity of injury, however, increased significantly with age ( $G = 96.65$ , 8 d.f.,  $P = 0.0001$ ). Age class I fish had the highest percentage of class "1" injury (17%), age III fish exhibited the highest percentages of the class "2" injury (27%), and age V fish the highest levels of class "3" injuries (29%), nearly triple that shown by age III and age IV fish (Figure 5).

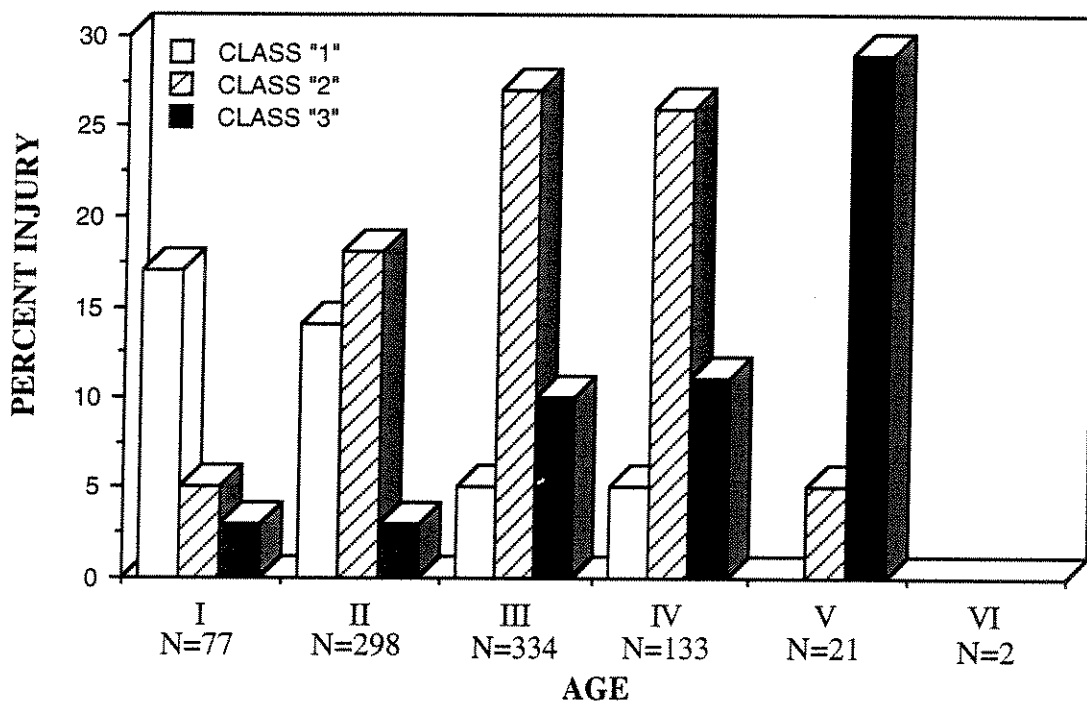


Figure 5. Incidence and severity of spinal injury by age class, all test fish combined. The two age VI fish were both uninjured and are represented as a blank.

Across all test groups, the combined mean length of injured fish ( $24.9 \text{ cm} \pm 3.9 \text{ cm}$ ) was not significantly different than uninjured fish ( $24.6 \text{ cm} \pm 0.19 \text{ cm}$ ) ( $U = 82361$ ,  $P = 0.11$ ). However, injury class "3" ( $26.7 \text{ cm} \pm 0.48 \text{ cm}$ ) and injury class "2" fish ( $25.4 \text{ cm} \pm 0.3 \text{ cm}$ ) were significantly longer than uninjured fish ( $U = 12344$ ,  $P \leq 0.01$ ;  $U = 42414.5$ ,  $P = \leq 0.01$ ). Uninjured fish were significantly longer than injury class "1" fish ( $22.4 \text{ cm} \pm 0.4 \text{ cm}$ ) ( $U = 15462.5$ ,  $P \leq 0.01$ ) (Figure 6).

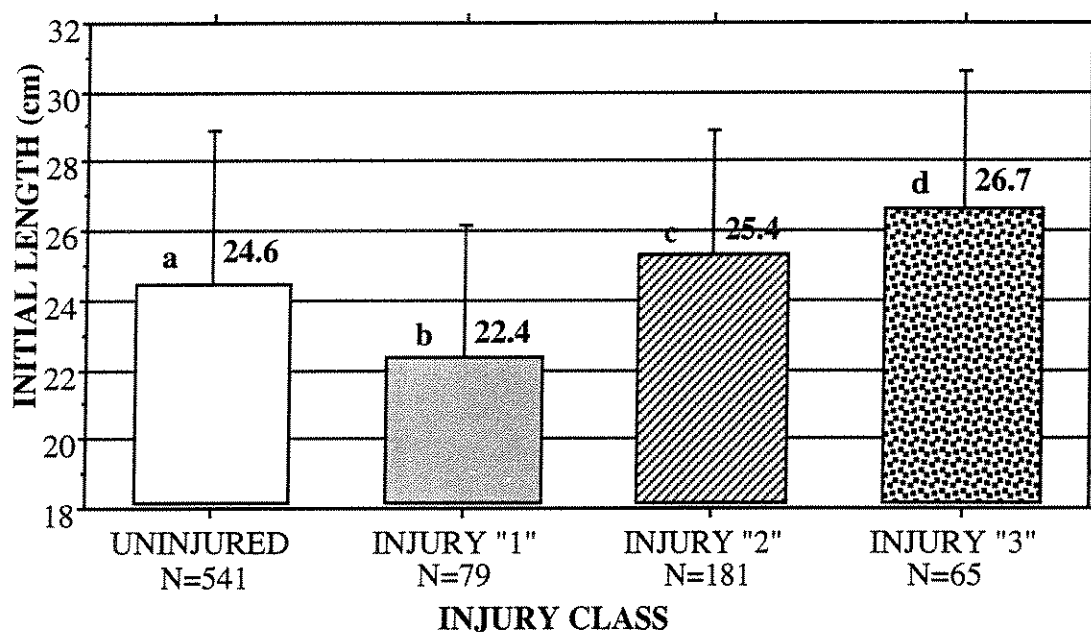


Figure 6. Mean length of rainbow trout in each spinal injury class. Differing small case letters indicate significant differences (95 % confidence level) between groups.

### Effects of Waveform on Growth and Condition

#### Short-term Growth and Condition

A sample of 13 - 14 % of each test group was removed from the pond 100 d post-release to assess short-term growth and condition. Growth and condition was assessed only for fish retaining the VI tag (VI tag loss was 39% SM, 68% HP and 30% FP).

Mean weight change of VI tagged fish was not significantly different between the three test groups (Figure 7;  $H = 3.85$ ,  $P = 0.15$ ). Fish collected from the SM group (22.6 g) however, did gain about 3 times that of the HP group (8.8 g) and nearly double the weight of FP fish (13.3 g).

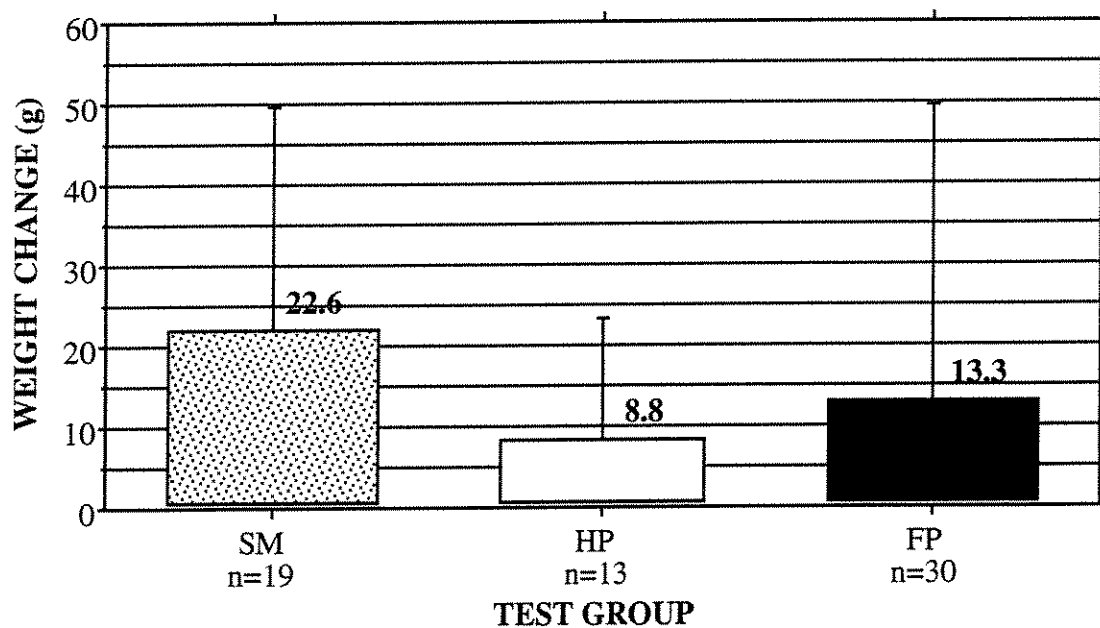


Figure 7. Mean weight change ( $\pm$  standard deviation) of rainbow trout from each test group 100 d post-release.

Change in mean length was also not significantly different (Figure 8;  $H=4.55$ ,  $P=0.10$ ). Smooth DC fish did however, show the greatest increase in length (1.7 cm), twice the increase of HP fish at 0.8 cm. Smooth DC and FP fish showed about an equal increase in length.

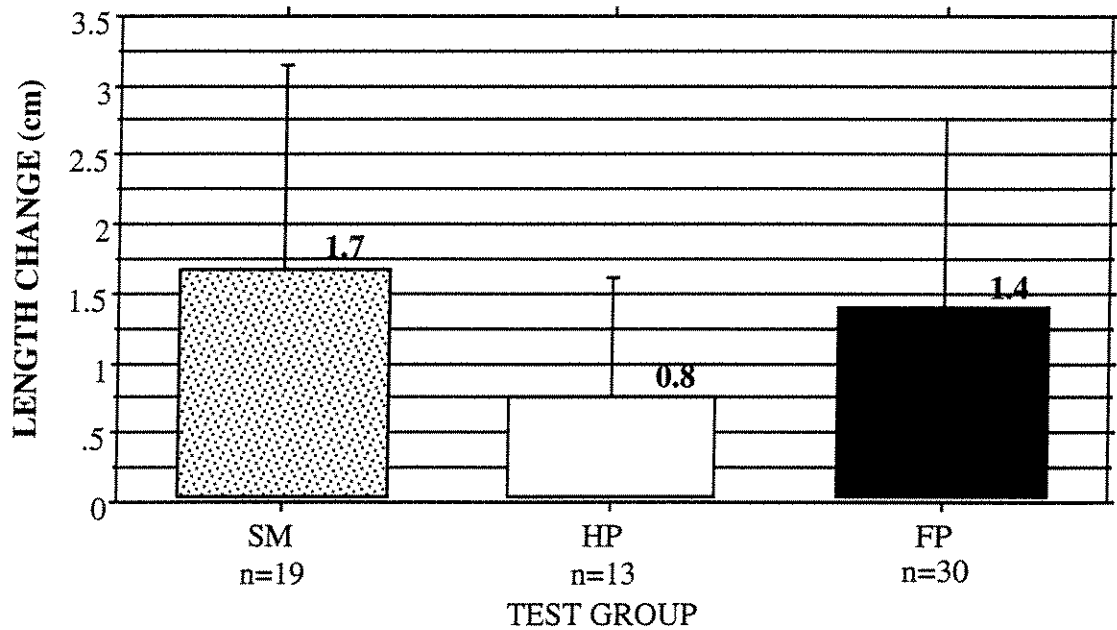


Figure 8. Mean length change ( $\pm$  standard deviation) of rainbow trout from each test group 100 d post-release.

The mean condition factor change showed no significant differences between the three test groups (Figure 9;  $H = 1.42$ ,  $P = 0.49$ ). Trout from all three test groups lost condition with FP fish demonstrating the greatest average reduction and greatest variation in condition.

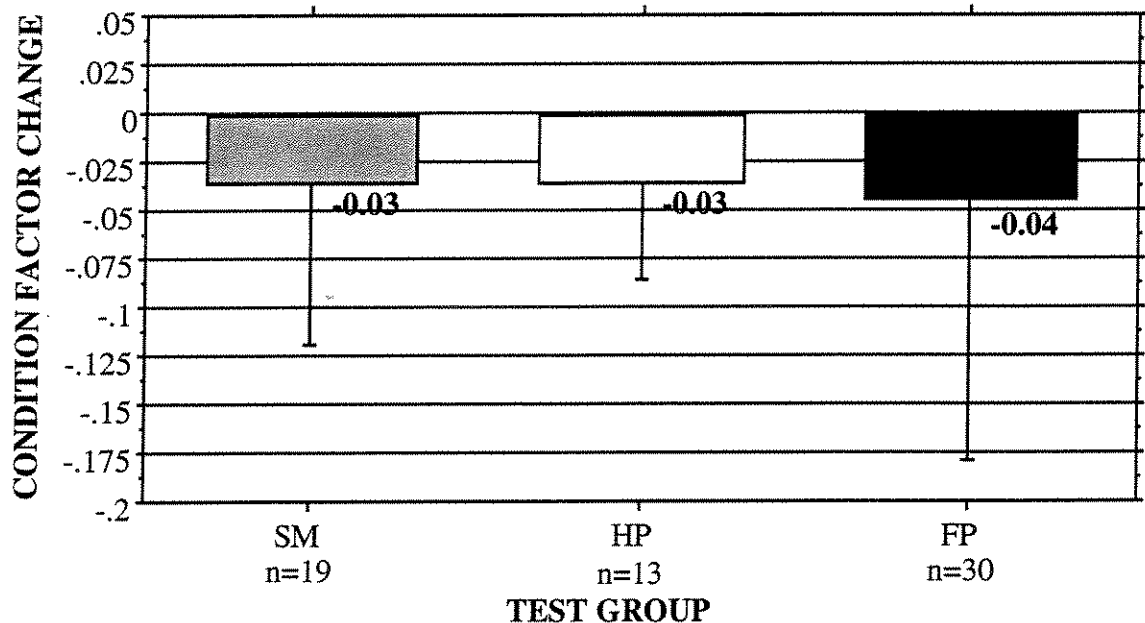


Figure 9. Mean condition factor change ( $\pm$  standard deviation) of rainbow trout from each test group 100 d post-release.

#### Long-Term Growth and Condition

Visible Implant tag loss remained high after 1 yr in all three test groups (35 - 63%). As a result, long-term growth and condition data were based on 54 SM, 57 HP and 103 FP fish.

In contrast to short-term growth, mean weight change after 1 yr in the pond was significantly different among the three test groups (Figure 10;  $H = 6.02$ ,  $P = 0.05$ ). All three test groups increased in weight but HP fish exhibited the greatest average gain at 45.9 g. Smooth DC fish gained significantly less weight than HP fish ( $U = 1167$ ,  $P = 0.03$ ) as did FP fish ( $U = 2361$ ,  $P = 0.03$ ). There was no significant difference in weight change between the SM and FP fish.

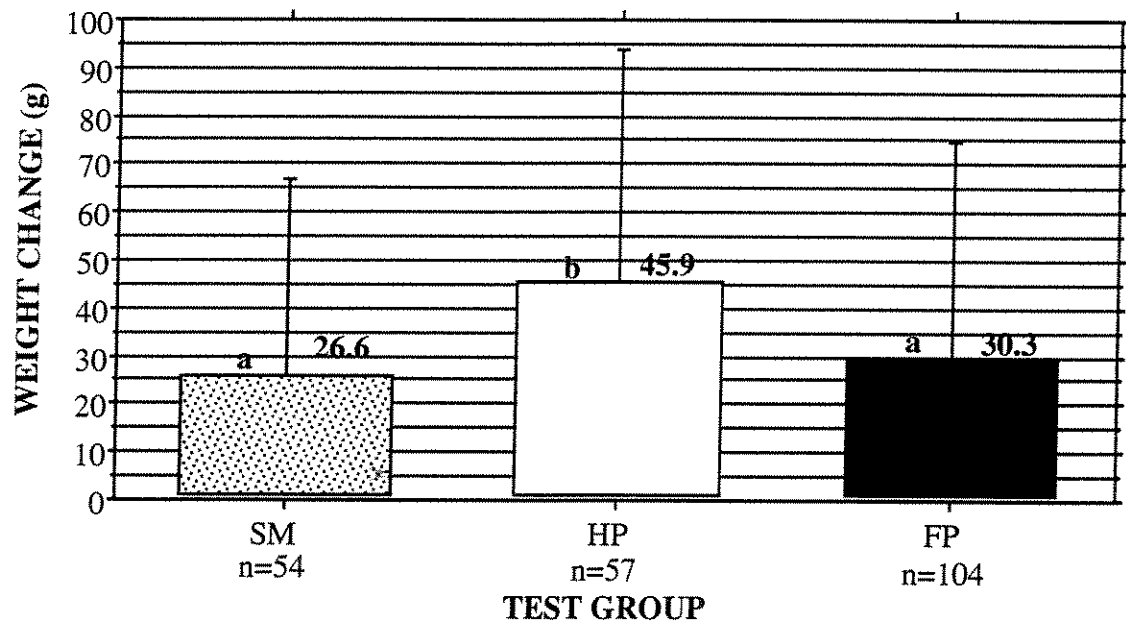


Figure 10. Mean weight change ( $\pm$  standard deviation) of rainbow trout 1 year after release. Differing small case letters indicate significant differences ( $P \leq 0.05$ ) between test groups.

Significant differences in mean length change also occurred (Figure 11;  $H = 5.95$ ,  $P = 0.05$ ) with the HP fish demonstrating the greatest average increase (2.0 cm). This was significantly greater than SM fish (0.9 cm) ( $U = 1137$ ,  $P = 0.02$ ) but not significantly greater than the FP fish (1.4 cm) ( $U = 2479$ ,  $P = 0.09$ ). There was no significant difference in the length change between the SM and FP fish or the HP and FP fish.

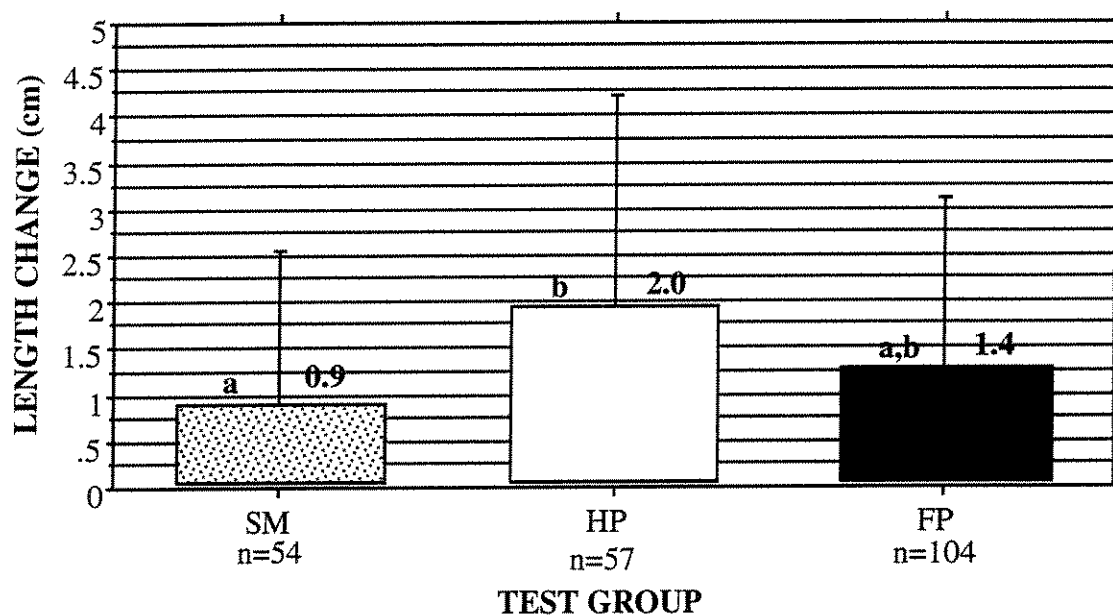


Figure 11. Mean length change ( $\pm$  standard deviation) of rainbow trout from each test group 1 year after release. Differing small case letters indicate significant differences ( $P \leq 0.05$ ) between test groups.

Mean condition factor change was not significantly different between the test groups (Figure 12;  $H=3.66$ ,  $P=0.16$ ). However, fish from all three test groups showed an increase in condition compared to the decrease observed at 100 d post-release.



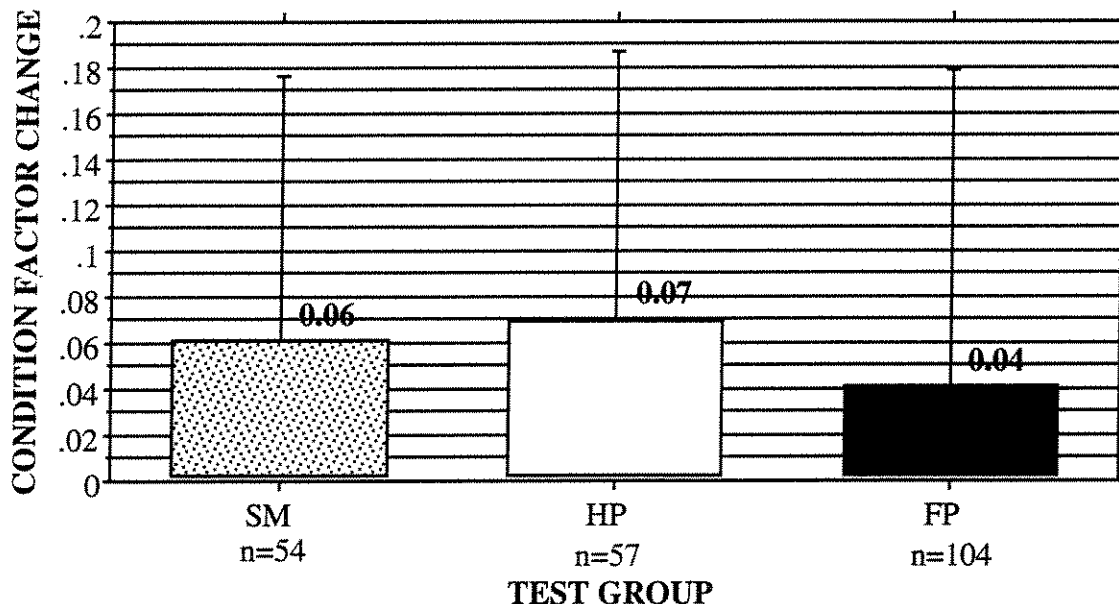


Figure 12. Mean condition factor change ( $\pm$  standard deviation) of rainbow trout from each test group 1 year after release.

### Effects of Injury on Growth and Condition

#### Short-term Growth and Condition

Despite a lack of difference in short-term growth between test groups, (p.17) there was a significant difference in weight change between uninjured and injured fish combined across test groups (Figure 13;  $H = 17.26$ ,  $P \leq 0.01$ ) after 100 d. Uninjured fish gained significantly more weight (25.5 g) than injury class "1" fish (12.8 g) ( $U = 53.5$ ,  $P \leq 0.01$ ) and class "2" fish (1.4 g) ( $U = 92$ ,  $P \leq 0.01$ ). Although fish of injury class "3" exhibited a decrease of -19.5 g, this was not significantly different ( $U = 120$ ,  $P = 0.07$ ) than uninjured fish because of small sample size and high variation in weight change. Injury class "1" fish did not gain significantly more weight than injury class "2" fish. There was, however, a significant difference in the amount of weight gained between uninjured fish and all injured fish combined ( $U = 265.5$ ,  $P \leq 0.01$ ).

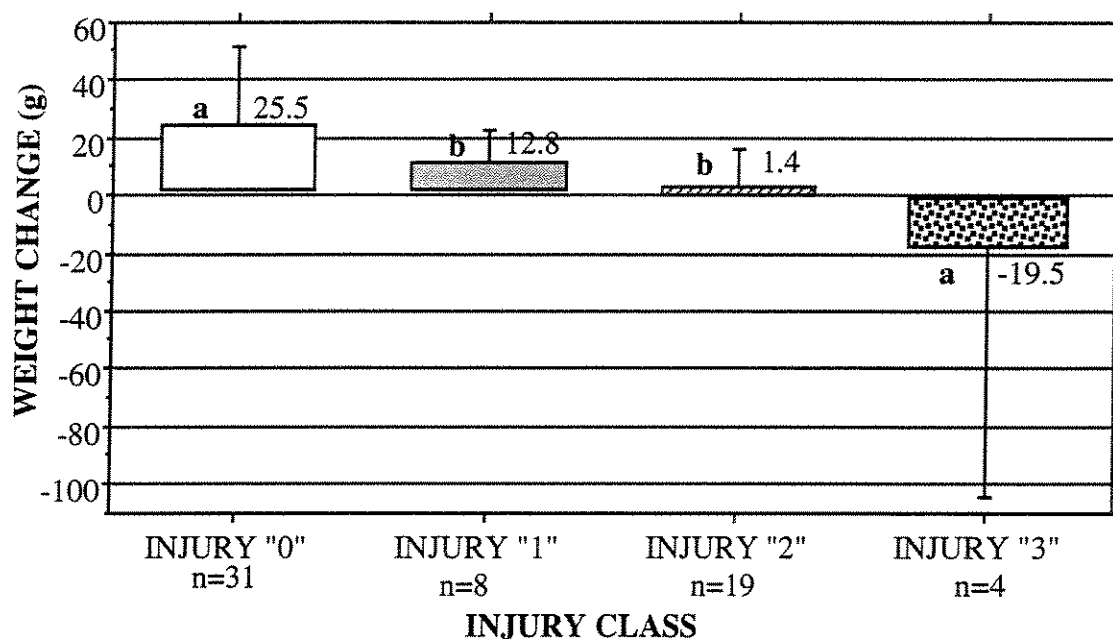


Figure 13. Mean weight change ( $\pm$  standard deviation) of rainbow trout by injury class 100 d post-release. Differing small case letters indicate significant ( $P \leq 0.05$ ) differences between injury classes.

Length change between the four injury classes was also significantly different (Figure 14;  $H = 16.81$ ,  $P \leq 0.01$ ). Uninjured fish gained an average of 2 cm which was not significantly greater than the length increase of injury class "1" fish (1.1 cm) ( $U = 72.5$ ,  $P = 0.07$ ) but was significantly greater than class "2" ( $U = 109.5$ ,  $P \leq 0.01$ ) and class "3" fish ( $U = 15.5$ ,  $P = 0.06$ ) which grew an average of 0.6 cm and 0.9 cm, respectively. There was also a significant difference in the average length gain of the uninjured fish and all injured fish combined ( $U = 197.5$ ,  $P \leq 0.01$ ).

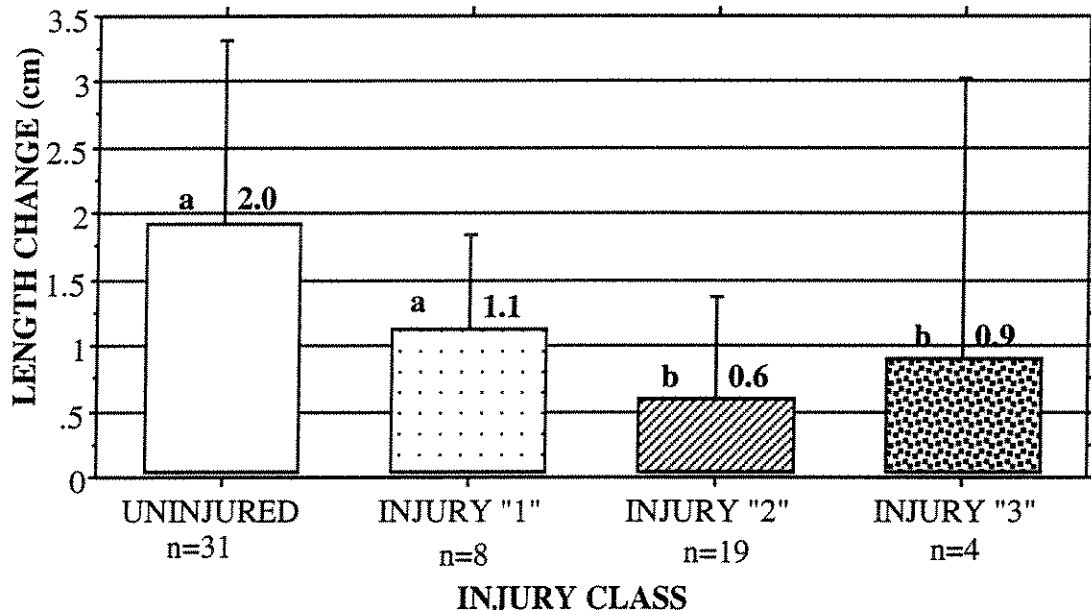


Figure 14. Mean length change ( $\pm$  standard deviation) of rainbow trout by injury class 100 d post-release. Differing small case letters indicate significant ( $P \leq 0.05$ ) differences between injury classes.

Condition factors varied significantly as well (Figure 15;  $H = 13.98$ ,  $P \leq 0.01$ ). While the average change in condition of uninjured fish was near zero, injured fish all exhibited a decrease in condition. The difference in condition factor change between injury class "2" (-0.048) and uninjured fish was significant ( $U = 122$ ,  $P \leq 0.01$ ), but no statistical difference occurred between uninjured fish and injury class "1" and "3" fish. As before, injury class "3" fish showed the highest variation. The reduction in condition factor of all injured fish combined was significantly greater than uninjured fish ( $U = 218.5$ ,  $P \leq 0.01$ ).

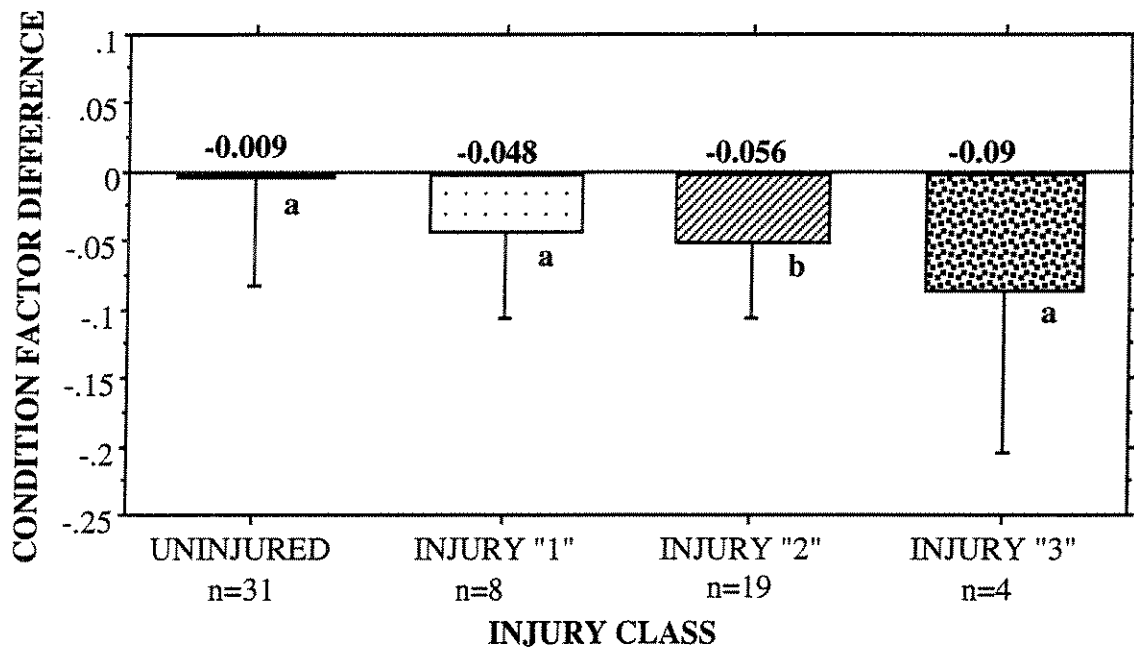


Figure 15. Mean condition factor change ( $\pm$  standard deviation) of rainbow trout by injury class 100 d post-release. Differing small case letters indicate significant ( $P \leq 0.05$ ) differences between injury classes.

#### Long-term Growth and Condition

Uninjured fish gained significantly more weight than fish which exhibited spinal injury of class "2" and "3" after 1 year in the pond (Figure 16). Uninjured fish gained an average of 44.3 g compared to a net loss of 4.5 g for the injury class "3" fish ( $U = 316$ ,  $P \leq 0.01$ ) while class "2" fish gained an average of 15.4 g. Injury class "1" fish gained an average of 36.9 g which was not significantly different than uninjured fish ( $U = 1481$ ,  $P = 0.48$ ). The three injury classes, when combined, showed a significantly lower weight increase than uninjured fish ( $U = 3645.5$ ,  $P \leq 0.01$ ).

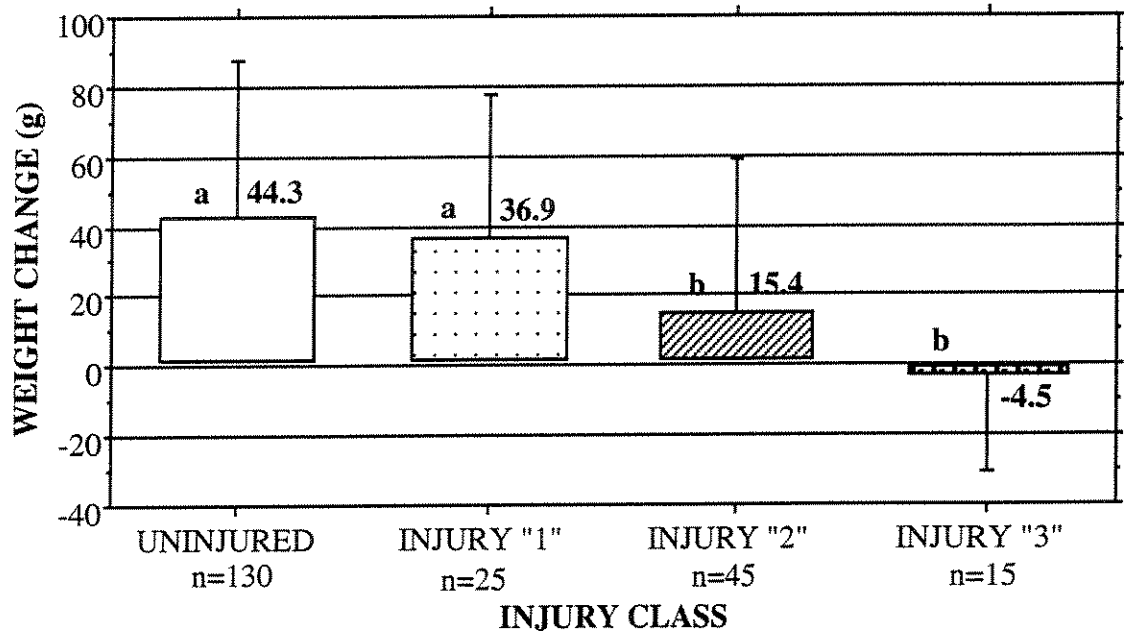


Figure 16. Mean weight change ( $\pm$  standard deviation) of rainbow trout by injury class 1 year after release. Differing small case letters indicate significant ( $P \leq 0.05$ ) differences between injury classes.

Similarly, uninjured fish grew significantly longer than fish exhibiting class "2" and class "3" injury ("2";  $U = 1879$ ,  $P \leq 0.01$ , "3";  $U = 487$ ,  $P \leq 0.01$ ). A significant difference in length was found among all injury classes ( $H = 25.22$ ,  $P \leq 0.01$ ) as well as between the uninjured and all injured fish combined (Figure 17;  $U = 4276$ ,  $P \leq 0.01$ ). As was the case with weight change, there was no significant difference in length between the uninjured fish and the injury class "1" fish ( $U = 1340$ ,  $P = 0.17$ ).

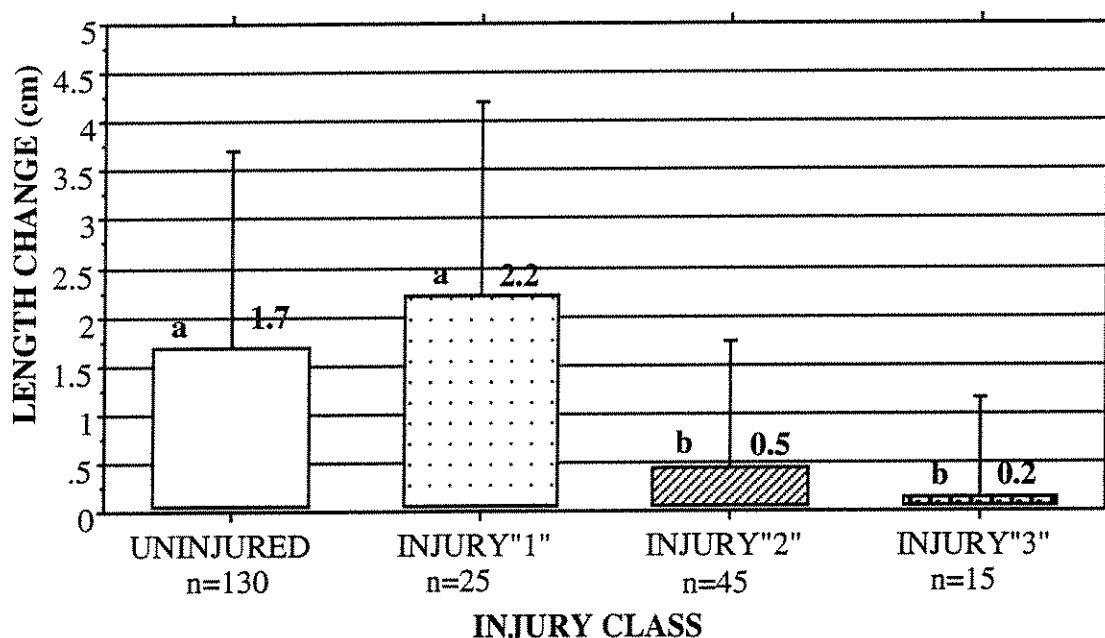


Figure 17. Mean length change ( $\pm$  standard deviation) of rainbow trout by injury class 1 year after release. Differing small case letters indicate significant ( $P \leq 0.05$ ) differences between injury classes.

Condition factor change was also significantly different between the injury classes after 1 year (Figure 18;  $H = 19.92$ ,  $P \leq 0.01$ ). The average condition of uninjured fish was significantly greater than fish exhibiting injury class "1" ( $U = 1076$ ,  $P \leq 0.01$ ), injury class "2" ( $U = 2353.5$ ,  $P = 0.05$ ) and injury class "3" fish ( $U = 268.5$ ,  $P \leq 0.01$ ). The greatest difference between condition factors occurred between uninjured fish and class "3" fish which showed a decrease of -0.03. Uninjured fish increased in condition significantly more than all fish from the three injury levels combined ( $U = 3698$ ,  $P \leq 0.01$ ).

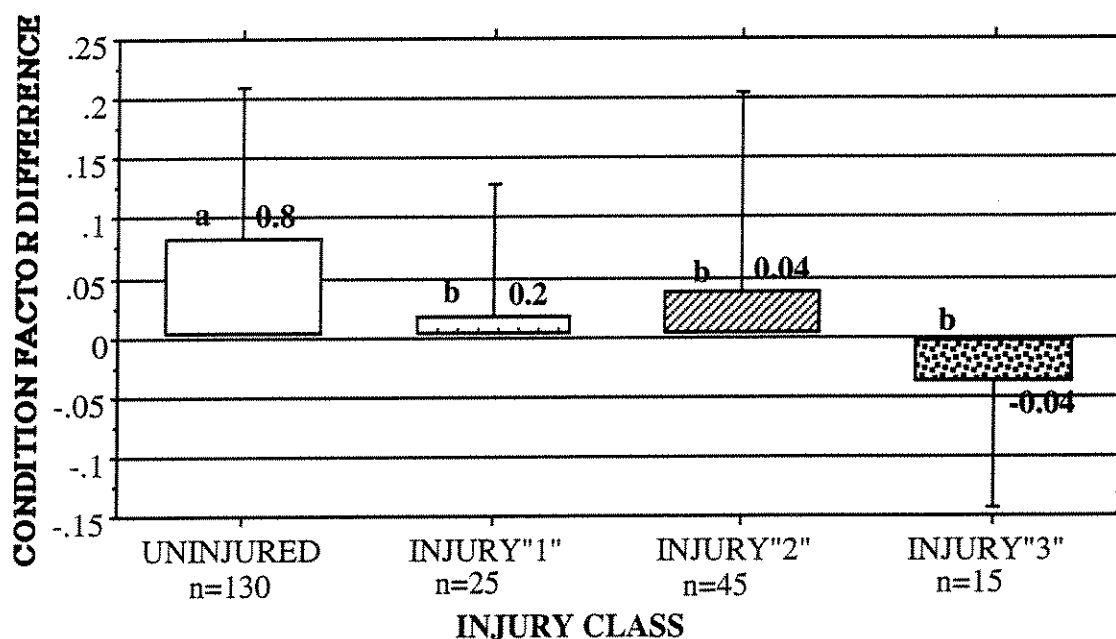


Figure 18. Mean condition factor change ( $\pm$  standard deviation) of rainbow trout by injury class 1 year after release. Differing small case letters indicate significant ( $P \leq 0.05$ ) differences between injury classes.

### Effects of Age on Long-term Growth

Fish from the three test groups were combined and analyzed for differential growth by age. Age I fish gained significantly more weight than Age III and Age IV fish (Figure 19;  $H = 77.94$ ,  $P \leq 0.01$ ) but not Age II fish ( $U = 347$ ,  $P = 0.07$ ). Age IV fish lost an average of 6.3 g which was significantly less than Age III fish (24.6 g) ( $U = 1035.5$ ,  $P \leq 0.01$ ) and Age II fish (62.1 g) ( $U = 147$ ,  $P \leq 0.01$ ). Not included in the weight change analysis was one uninjured age V fish which lost 92.4 g.

Similarly, age I fish grew significantly longer (4.9 cm) than all other age classes (Figure 20;  $H = 131.6$ ,  $P \leq 0.01$ ). Age II fish (2.8 cm) grew significantly more than age III fish ( $U = 723$ ,  $P \leq 0.01$ ) and age IV fish ( $U = 33$ ,  $P \leq 0.01$ ).

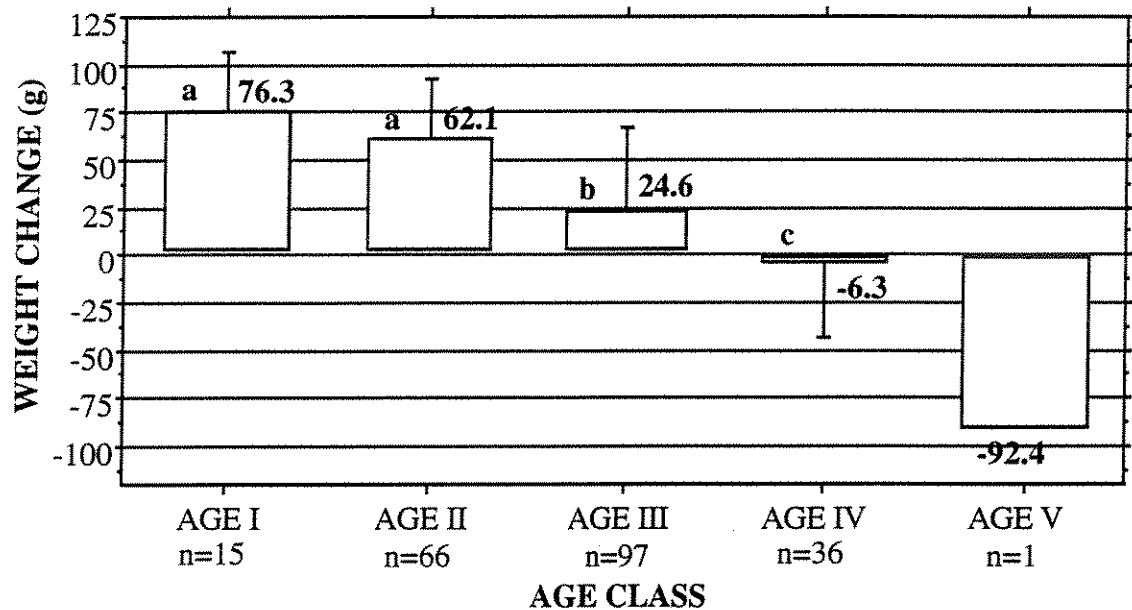


Figure 19. Mean weight change ( $\pm$  standard deviation) by age of rainbow trout 1 year after release. Trout from the three test groups were combined and sorted by age. Differing small case letters indicate significant ( $P \leq 0.05$ ) differences between injury classes.

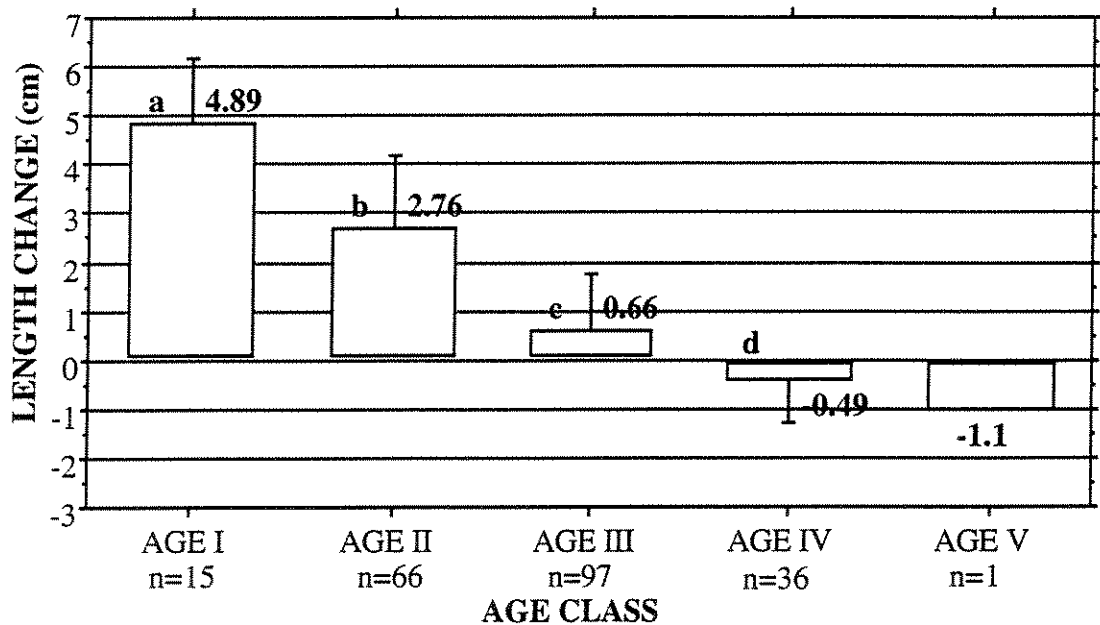


Figure 20. Mean length change ( $\pm$  standard deviation) by age of rainbow trout 1 year after release. The single age V fish was not included in statistical analysis. Differing small case letters indicate significant ( $P \leq 0.05$ ) differences between injury classes.



Long-term growth of fish in this study was compared to the estimated annual growth of wild rainbow trout in the Gallatin River (Figure 21). Age class I and II fish from the pond gained more than same age river fish. Conversely, age III and IV pond fish gained less than age III and IV river fish. Length change followed the same trend with age I (4.9 cm) pond fish growing more than age I river fish (1.6 cm) while age II fish from both pond and river grew the same (2.8 cm). Age III and IV river fish (2.9 cm and 0.99 cm) grew more than same age pond fish (III = -0.5 cm, IV = -1.1 cm).

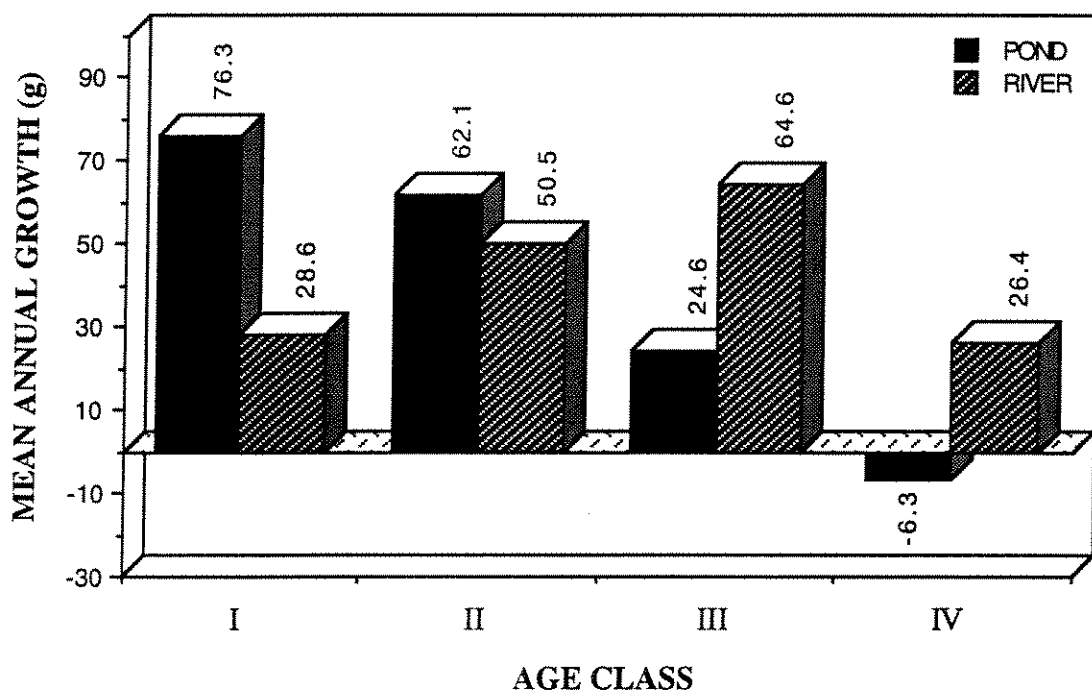


Figure 21. Mean annual growth by age of wild rainbow trout from the MSU study pond and the Gallatin River. Pond fish growth is compared to growth data (1983-84) for river fish (M. Lere, MDFWP, pers. comm.).

## EFFECTS OF WAVEFORM AND INJURY ON MORTALITY

### Short-term Mortality

One HP fish died in transit to the pond. This mortality was not electrofishing related and was not included in the short-term mortality data.

Short-term mortality (7 d post-release) was low among all test groups, ranging from 0.6 to 5.8% but was significantly different between groups. (Figure 22;  $G = 15.4$ , 2 d.f.,  $P \leq 0.01$ ). The highest mortality occurred in the SM group with 12 fish collected while only one fish was collected from the FP group and two from the HP group. The significantly higher mortality rate recorded for the SM group was likely due to the escape of 83 SM individuals from the raceway and the resultant extra handling and associated stress required to recapture them.

Only 21 mortalities were recovered from the pond. Of these, 75 % were recovered within the first week following release (Figure 22). Avian scavengers were observed removing some mortalities from the pond, which may have limited the number of recoveries after this period.

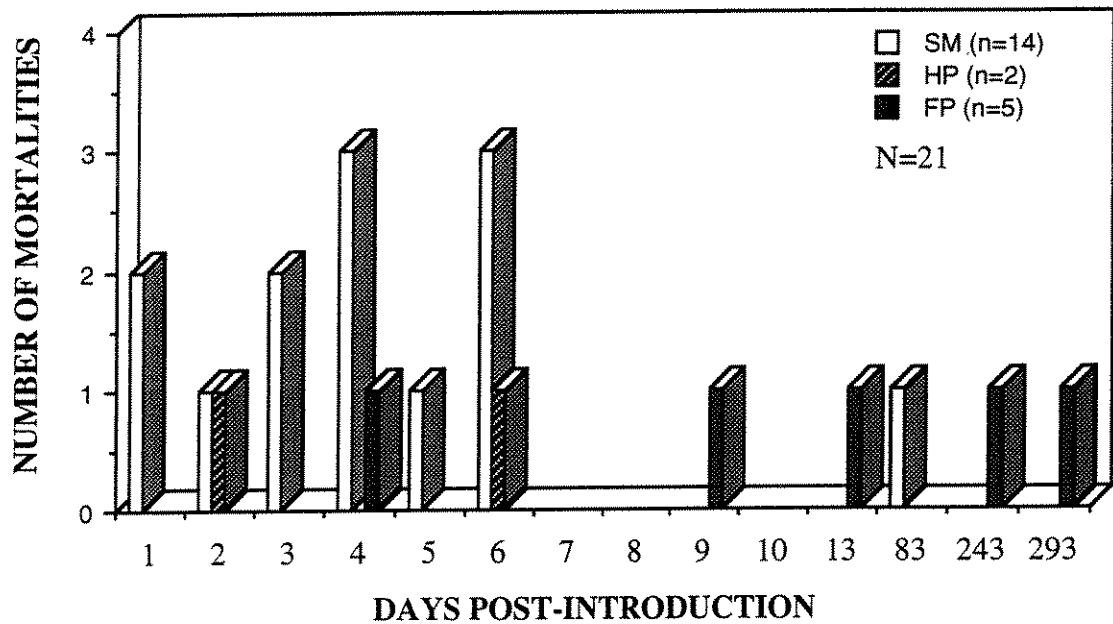


Figure 22. Number of mortalities collected per day from the three test groups. No mortalities were collected from the pond during the period not represented on the figure although it was patrolled during this period.

I was unable to evaluate the relationship between mortality and spinal injury because sample sizes were small ( $n=10$ ) due to high tag loss (48% of 21 mortalities collected). Seven of the nine mortalities collected with VI tag did not exhibit any initial electrofishing-induced spinal injury whereas two of the nine fish exhibited injury, one class "2" and one class "3".

### Long-term Survival by Waveform

Survival after 1 year ranged from 54 % for SM to 58 and 60 % for HP and FP, respectively (Figure 23). Differences in long-term survival were not significant between the three test groups ( $G = 0.341$ , 2 d.f.,  $P = 0.84$ ).

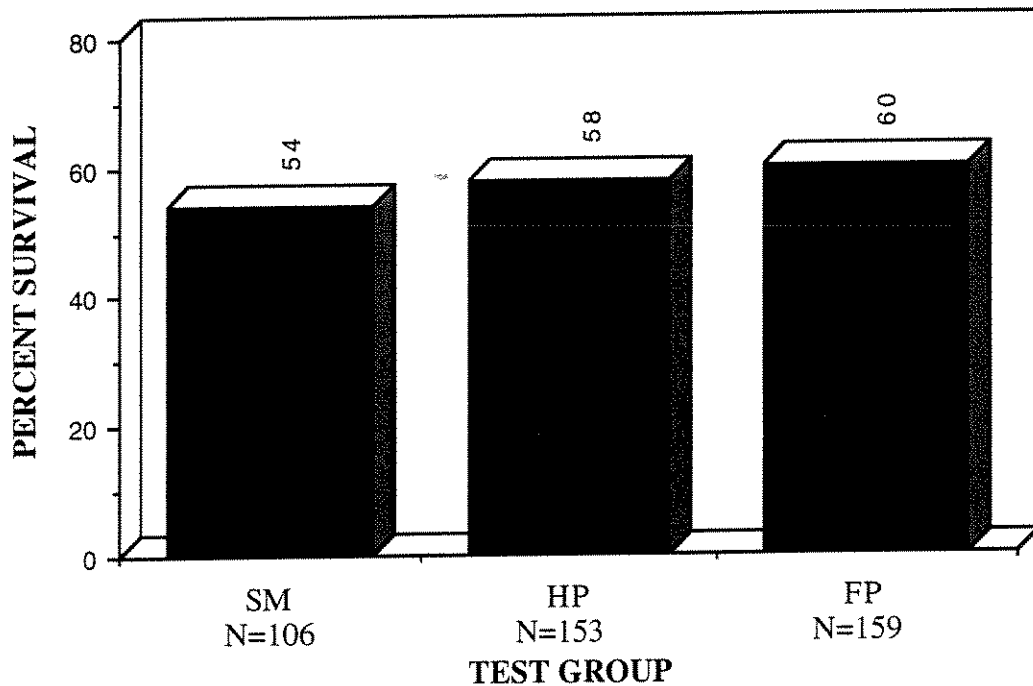


Figure 23. Percent survival of the three test groups 1 year after release.

### Long-term Survival by Injury Class

There was no significant difference in the injury composition of 1 year survivors when compared to the initial injury composition by test group (Figure 23; SM,  $G = 0.50$ ,  $P = 0.92$ ; HP,  $G = 2.30$ ,  $P = 0.53$ ; FP,  $G = 0.30$ ,  $P = 0.96$ ). There was also no significant difference in the long-term survival of uninjured fish compared to fish from all injury

classes combined (SM;  $G = 0.5$ , 1 d.f.,  $P = 0.83$ , HP;  $G = 0.02$ , 1 d.f.,  $P = 0.89$ , FP;  $G = 0.02$ , 1 d.f.,  $P = 0.89$ ).

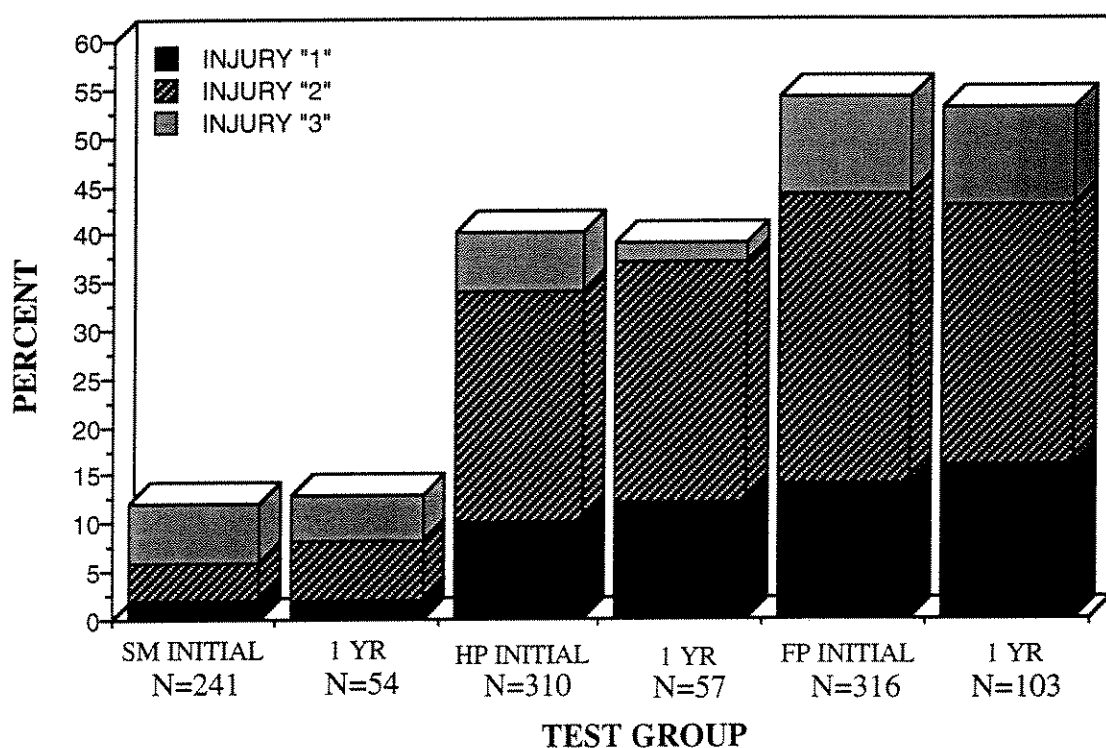


Figure 24. Initial injury composition compared to composition of 1 year survivors (VI tagged) by test group.

### Healing of Spinal Injuries

Spinal healing was evaluated for 38 surviving fish that had retained the VI tag and had sustained electrofishing injuries. Spinal injury rating increased in severity for 23 fish, remained unchanged for 13 fish and decreased in 2 fish (Figure 25). Calcification of individual vertebrae was more apparent for severely injured vertebrae, which caused the upgrade in severity in the majority of cases. In several fish, injury was not detected on the initial x-ray but after one year, calcification was present and injury apparent. Furthermore, some fish that contained initial injury rating of "1" were rated as "3" following 1 year

suggesting that hairline fractures were present but not detected. In general, the integrity of injured vertebra after 1 year appeared to be reduced or lost and in many cases, individual injured vertebra could no longer be distinguished.

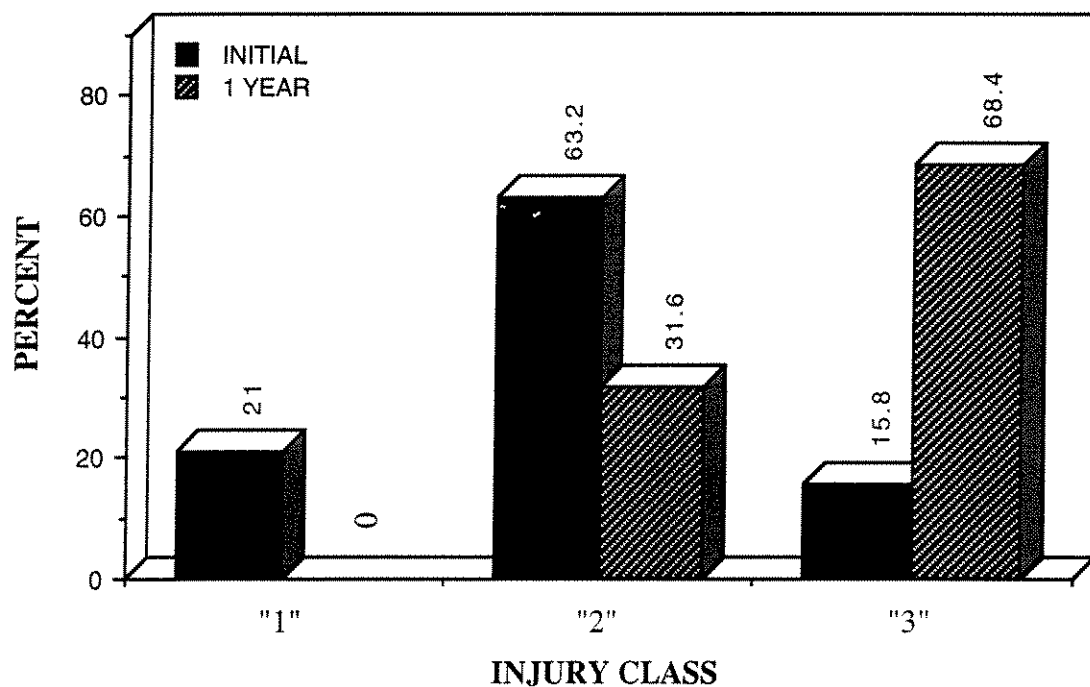


Figure 25. Initial and 1 year composition of spinal injury after fish were rated for healing.

## DISCUSSION

Initial injury rates of the wild rainbow trout collected with three DC waveforms were similar to those established for wild rainbow trout by Sharber and Carothers (1988) and hatchery rainbow trout by McMichael (1991). Injury rates were highest in the Full pulse DC group at 54 %, followed by Half pulse DC and Smooth DC at 40 % and 12 % respectively. Similarly, Fredenberg (1992), using the same equipment on the Madison River, Montana, documented markedly higher rates of spinal injury in wild rainbow trout electrofished with FP (73.3 %) followed by HP (63.8 %) and SM (30.4 %). Recently, higher injury rates with increased voltage and pulse frequencies were observed for hatchery rainbow trout by Taube (1992). My study corroborates previous findings that incidence of spinal injury increases with pulse frequency. Furthermore, my findings and those of Fredenberg represent further evidence that wild fish are more susceptible to injury from electric fields than hatchery fish (Woodward and Strange 1987; Mesa and Schreck 1989).

Even though there were significant differences in overall injury rate, there was no significant difference in the rate of injury class "3" induced by the three waveforms (SM = 6 %, HP = 6 %, FP = 10 %). This same trend was observed by Fredenberg (1992) who noted that the greatest variation in injury between the same three DC waveforms used in this study occurred within injury class "2", while rates of injury class "3" remained relatively constant (SM = 5.4 %, HP = 10.6 %, FP = 13.3 %). The average power gradient of the three waveforms at short distances from the anode (< 25 cm) (Figure 2) were found to be similar. Fish netted in this area of high field strength are theorized to demonstrate the most severe response (partial or full tetanus) which may result in a similar incidence of class "3" spinal injury (Snyder 1992).

Severity of electrofishing-induced injury increased significantly with length and estimated age of wild rainbow trout while incidence did not. Age V fish (28.8 - 32.8 cm F.L.) exhibited nearly triple the rate of class "3" injury compared to age III (19.0 - 24 cm) and IV (24.1 - 28.7 cm) fish and an approximate tenfold increase over age I (< 14.6 cm) and II fish (14.6 - 18.9 cm). This study differs from recent studies in that samples of fish were not restricted to large fish (>30 cm). Spinal injury in larger fish is more easily quantified due to heavier calcification of bony tissue, but with the excellent definition provided by x-ray equipment used on this study, injury was believed to be accurately quantified in nearly all fish longer than 15 cm. The higher incidence of severe injury in larger fish conforms with current theory that larger fish appear to be more sensitive to electrical currents than small fish (Sullivan 1956; Ellis 1975; Sharber and Carothers 1988; Cowx and Lamarque 1990; Reynolds 1993). Conversely, Fredenberg (1992) and Taube (1992) failed to show a length/injury relationship under the conditions of their experiments, as these studies looked only at incidence rather than severity of spinal injury.

Long-term and short-term growth and condition of wild rainbow trout were not significantly influenced by waveform. Whether a nonelectrofished control group would have exhibited similar impacts on growth remains in question as collection of such a group was logistically beyond the realm of this study. Although VI tag loss limited sample sizes, short-term reductions in growth and condition were observed in the FP and HP groups when compared to SM. However, this trend was not carried over to the long-term where growth and condition increases were significantly greater in the HP group. In previous studies, single pass electrofishing has failed to reveal adverse effects on growth (Snyder 1992). However, repeated electrofishing events (twice within a 1 to 3 d period at intervals of 1.5 to 7 months) have been shown to reduce short and long-term weight gain in rainbow and brown trout (Gatz et al. 1986). Presumably, repeated electrofishing would have the effect of increasing incidence and severity of injury although fish were not x-rayed or



necropsied in the Gatz et al. study. Taube (1992) found no difference in long-term growth (203 d) involving hatchery rainbow trout although he noted that the results could be quite different in a study involving wild fish and higher water conductivities.

Uninjured fish grew significantly more than injured fish at both short and long-term intervals. Spinal injury was associated with a significant reduction in both short and long-term growth with short-term growth reductions observed in class "3" fish (-19.5 g) compared to uninjured fish who gained 25.5 g. Long-term growth of class "3" fish also showed a weight loss of -4.5 g while class "2" (15.4 g) and class "1" (36.9 g) and uninjured fish (44.3 g) all gained weight. My results suggest that the negative impacts of electrofishing appear to be strongly influenced by the severity of injury rather than exposure to electrical current alone. This study is unique in the respect that growth and survival are tested specifically against severity of injury as well as waveform.

Age structure of the 65 fish from the three test groups that exhibited class "3" injury may have influenced the average long-term growth rates. Spinal injury increased in severity with age resulting in more growth in age I and II than age III and IV fish while the 36 age IV fish lost weight. The same significant trend was observed in uninjured, shocked fish from the three test groups. Uninjured age I and II fish gained an average of 83.2 g and 68.4 g compared to age III fish (40 g) and age IV fish (0.9 g) suggesting that pond related factors as well as electrofishing injury played a role in growth. In the only other study involving growth by age classes, Gatz et al. (1986) discovered that instantaneous growth rates of rainbow trout shocked repeatedly over a 1 year period with pulsed DC were reduced more significantly in age I and II fish than among age III fish.

Long-term survival was not affected by severity of electrofishing-induced spinal injury or waveform. Injury composition of surviving fish was not significantly different than the initial injury composition for each test group. One year survival ranged from 54 % to 60 % for the three test groups. SM fish recorded the lowest survival (54 %) although

this was not significantly less than the HP group (58 %) or the FP group (60 %). This verifies many studies that have failed to show an adverse impact on survival in fish collected with single pass electrofishing using various PDC waveforms (Snyder 1992). Recently however, Taube (1992) found significant differences in long-term mortality (203 d) between shocked-injured (52 %), shocked-uninjured (29 %) and unshocked control (10 %) groups in hatchery rainbow trout. These fish were shocked in comparatively low conductivity water (100 - 121  $\mu\text{S}/\text{cm}$ ) and under a controlled setting (homogeneous field) where fish could not escape as they would in the wild. Furthermore, he found no significant difference in mortality between test groups when fish were allowed to avoid high current densities. Fish collected from the wild have the ability to avoid electrical current which may influence initial stress or injury levels and translate into lower mortality than electroshocking under controlled conditions in the hatchery.

This experiment is unique in the respect that wild riverine fish were monitored for mortality for 1 year in a pond environment. Many electrofishing studies, looking at impacts on salmonids, have dealt with hatchery fish or have monitored mortality over relatively short duration (< 30 d). Therefore questions remain regarding the applicability of these data to electrofished populations that remain in the stream. Stresses incurred on wild fish during annual population estimates may be lower than during this experiment because fish are not affected by the excess handling associated with the transport, double tagging and x-raying. Conversely, growth and survival impacts may be more pronounced in fishes remaining in the stream environment because of the presumably higher energetic demands associated with capturing food and maintaining positions in flowing water. In the wild, reduced growth of severely injured fish (larger fish) may affect competitive ability thus interfering with the social hierarchy of stream fishes (Mesa and Schreck 1989).

Growth rates of age class I and II fish in the pond were greater than rainbow trout in the Gallatin River while age classes III and IV were less. This suggests that younger

fish were more adaptable to the pond environment and out competed older fish for available food. Furthermore, large prey items may have been reduced early in the experiment allowing an advantage to the smaller fish. Fish were stocked in the pond at comparatively high densities (1420 fish per ha) which may have further impacted age classes differentially. Age I pond fish (76.3 g) grew more than all age classes in the pond compared to age III river fish (63.6 g) which grew the most. This shift could be due to the change in environment but also, age I fish had the lowest incidence of injury (25 %) of the five age classes.

Spinal injuries appeared to increase in severity, due to calcification, over the year period of this experiment. The severity increased in the majority of fish examined because the calcification of injured vertebrae made injuries more pronounced. For example, fish that showed an initial injury rating of class "1" (21 %) (compression of vertebrae) were upgraded to class "2" injury (31.6 %) (misalignment of vertebrae) because calcification created an area that is misaligned. Healing may, in some cases, involve the fusing of vertebrae but it is not possible to determine this from x-rays alone. Several fish were observed with a string of fused vertebrae (10 - 15) that could not be distinguished from one another and that were not initially rated or rated as minor injuries. Calcification occurred in several fish that did not appear to have any spinal injury, suggesting that initial assessment of spinal injury may be underestimated using standard x-ray equipment.

Many studies have documented the injury rates that various waveforms produce under a variety of electrofishing situations and frequently, recommendations have been made regarding the future use of specific waveforms based primarily on injury rates (Cowx and Lamarque 1990; Fredenberg 1992; Taube 1992). Snyder (1992) recommended that to minimize injury, the use of direct current should be as smooth as possible. The use of low frequency, direct current waveforms does reduce the total injury rates. But, based on the results of Fredenberg (1992) and this study, the incidence of class "3" spinal injury appears

to remain relatively constant with varying pulse frequency. Therefore, the focus of future research dealing with the reduction of electrofishing-induced spinal injury should be to test waveforms and equipment variables that reduce or eliminate class "3" spinal injury.

Further considerations by management agencies regarding the impacts of electrofishing-induced injury should also include the perceptions of the public. In a recent creel survey where several thousand anglers were interviewed on the Bighorn River, Montana, many believed that all fish with observable spinal deformities are victims of electrofishing by the MDFWP (S. Dalbey, pers. obser.). Since spinal injuries may result from genetic or developmental processes (McCrimmon and Bidgood 1965), public education about electrofishing and its effects on fish is an important management issue.

The literature contains many examples of electrofishing studies that have attempted to determine the short-term and long-term impacts on growth and survival of various fish species. Even with standardized documentation of electrofishing variables in mind, we still observe highly variable injury rates caused under various electrofishing scenarios. Because of this, it is important to keep in mind that a very small percentage of these injuries are externally visible. Sharber and Carothers (1988: 118) stated that "X-ray analysis was the ultimate diagnostic technique for determining injury" and (1988: 121) "spinal injury can only be determined by x-ray or autopsy analysis". It is for these reasons that the importance of x-raying and quantifying the effects of spinal injury, especially in fish identified as sensitive to electrofishing, become higher priority in fisheries field work to further reduce impacts on wild trout populations we manage.

Electrofishing guidelines for the State of Montana, as proposed by Fredenberg (1992), are validated by the findings of my experiment. When electrofishing trout (rainbow, cutthroat and brown), smooth DC should be used whenever possible and pulse frequencies above 30 Hz should be avoided to minimize injury rates. Fish should be netted from the electrical field as quickly as possible to avoid prolonged exposure. Precautions

such as efficient netting and the avoidance of "double dipping" may go a long way towards reducing injury, especially the more severe (class "2" and "3") spinal injuries.

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