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Healing of Electroshock-Induced Hemorrhages in Hatchery Rainbow Trout

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Abstract.—We monitored healing in electroshock-induced hemorrhages of myomere blood vessels produced by individually exposing hatchery rainbow trout *Oncorhynchus mykiss* to direct current ($N = 502$) and pulsed direct current ($N = 708$). We used voltage gradients and exposure times that were suspected to produce high injury rates to facilitate observation of injury duration in muscle tissue. At 1 d postexposure, 86.1% of the test fish exposed to DC and 81.6% of those exposed to pulsed direct current (PDC) had at least one hemorrhage. Fish exposed to DC averaged 1.86 injuries at 1 d postshocking, and those exposed to PDC averaged 1.45 injuries. Number of hemorrhage injuries per fish began declining by 15 d postshocking in both groups. The severity of injuries initially increased through 15 d postshocking and then decreased through the remaining 3–5 weeks of the tests. At the end of the test, injuries induced by DC had declined by 78.0% (36 d postshocking), and those induced by PDC declined by 92.4% (57 d postshocking). In all, 1.8% of all fish exposed to DC and 1.1% of those exposed to PDC died during the study. Our data for hatchery rainbow trout suggest that hemorrhage injuries in salmonids caused by electrofishing exposure exist for a relatively short time and do not represent a long-term mortality or health risk to the fish. Because of the ephemeral nature of blood vessel hemorrhages, compared with spinal injuries, future studies that examine electrofishing injuries should evaluate hemorrhage and spinal injuries separately and abandon the practice of combining these data.

Electrofishing is an effective sampling tool for managing stream salmonids and other fish species and has been widely used since the 1950s and 1960s (Reynolds 1996). Recently, concerns about injuries induced by electrofishing have resulted in studies quantifying injury levels for a variety of electrical currents and wave forms (Sharber and Carothers 1988; Mesa and Schreck 1989; Fredenberg 1992; Dalbey et al. 1996). Injury typically occurs as spinal damage (e.g., vertebral compressions, separation of vertebrae, and fractures) or hemorrhage in muscle blood vessels (Reynolds 1996). Although neither injury is always externally apparent, both injuries are detectable using X-ray and necropsy methods.

Reynolds (1996) describes a visual rating system, first developed with participant input at a 1991 American Fisheries Society symposium, that ranks spinal and hemorrhage injuries from electrofishing on a scale from 0 to 3. Fredenberg (1992) used this system to evaluate electrofishing injuries in samples of salmonids collected from several waters in Montana but subsequently reported an overall injury rating for each fish based on the highest rank observed for either type of injury. For example, a fish with a level-3 hemorrhage injury and a level-2 spinal injury would be assigned a single level-3 injury. This worst-case system was used in several subsequent studies (McMichael 1993; Cowdell and Valdez 1994; Hollender and Carline 1994) but was not used by Thompson et al. (1997), who kept the rating scales separate.

Schill and Beland (1995) questioned the practice of lumping skeletal and muscle injuries because the latter are intuitively more likely to heal with less long-term effects. Lumping the two injury types, in essence, equates a severe and complete fracture of one or more vertebrae with the presence of a 2–3-mm blood vessel hemorrhage in muscle. If hemorrhages heal in a short period, it may be inappropriate to equate them with spinal injuries when evaluating the impacts of electrofishing. We are unaware of any study that has evaluated how long electrically induced hemorrhages persist in salmonids or other fish species.

The objective of this study was to document, at fixed time intervals, the quantity and severity of myomere blood vessel hemorrhages in hatchery rainbow trout *Oncorhynchus mykiss* after being electroshocked. Our general approach was to expose individual fish to the same electrofishing field, randomly assign them to treatment groups, and necropsy the treatment groups at fixed postshocking time intervals to quantify healing.

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Methods

Electrical exposure.—We exposed individual hatchery rainbow trout to continuous DC in 1996 and pulsed direct current (PDC) in 1998. Electrical exposures were conducted at the Nampa Fish Hatchery (Idaho Department of Fish and Game). A Coffelt model 15 VVP powered by a Honda 5,000-W generator provided electrical currents. The anode consisted of a 1-m-diameter Wisconsin ring with six droppers. The cathode was a 3-m length of flexible electrical conduit (5-cm diameter) lying on the bottom of the raceway. Test fish were individually placed in a dip net (0.46 × 0.38 m) immediately adjacent to, but not touching, the anode at the edge of a raceway wall. Fish were placed into the center of the net at the water surface, and the field was immediately activated. During the shocking period, fish could occupy any portion of the net from the surface down to a depth of 18 cm. Exposure was regulated with a foot switch and stop watch. Each fish was exposed to the electrical current for 5 s.

On November 25, 1996, we individually exposed 502 hatchery rainbow to DC at 375 V and 7.5 A. A random sample of all fish shocked averaged 264 mm in total length (SD = 26, N = 101). Electrical output readings were obtained using a 100-Hz analog oscilloscope (Hewlett-Packard model 1740A). Voltage gradient within the dip net, measured at the conclusion of the DC trial, ranged from 1.7 V/cm nearest the anode to 0.7 V/cm farthest from the anode. Water conductivity was 390 μs/cm at 12°C.

On March 12, 1998, we individually exposed 708 fish (mean total length = 287 mm, SD = 38, N = 103) to square-wave PDC current at 325 V (peak), 60 Hz (25% duty cycle), and 7.5 A (average). Voltage gradients within the dip net, measured at the conclusion of the PDC trial, ranged from 1.9 V/cm nearest the anode to 0.65 V/cm farthest from the anode. Water conductivity was 405 μs/cm at 14°C. The additional 200 fish used during 1998 allowed us to extend the observation period for several weeks longer than in the previous trial.

Following exposure, individual fish were removed from the dip net and placed into five or seven holding pens (depending on the year), in an adjacent hatchery raceway. Approximately 100 electrofished rainbow trout were randomly assigned on an individual basis to each holding pen to avoid sampling bias. Fish were fed a maintenance diet until sacrificed for necropsy. Test lots of rainbow trout exposed to DC were sacrificed at 1, 8, 15, 22, and 36 d postshocking. Test lots exposed to PDC were sacrificed at 1, 8, 15, 22, 29, 43, and 57 d postshocking.

Control groups of approximately 50 rainbow trout were handled identically to test fish during both DC and PDC trials but were not electroshocked. Controls were held in a live box (1.2 × 1.2 × 2.4 m) and sacrificed 1 d after handling to determine baseline levels of hemorrhages attributable to handling or natural occurrences from the hatchery environment. Raceway water temperature was monitored hourly with a recording thermograph during both trials.

Necropsies and injury ratings.—Before necropsy, all rainbow trout were given an overdose of tricaine methanesulfonate. To minimize bleeding during necropsy, in 1996 we cooled specimens (Reynolds 1996) by refrigerating them for 24 h; in 1998 we chilled the fish in a freezer 6 h. The left and right sides of specimens were filleted flush with the spinal column using an electric knife, and both fillets and the skeleton section were washed to remove any fresh blood. Each fillet and skeletal section was backlighted with a 60-W light bulb to increase hemorrhage visibility. For consistency, one of us filleted all study fish and the other evaluated all injuries during both trials. We ranked all observed hemorrhages for each fish on a scale of 0 to 3, where 0 = no hemorrhage, 1 = wound separate from spine, 2 = wound on spine less than or equal to the width of two vertebrae; and 3 = wound on spine greater than the width of two vertebrae (Reynolds 1996). Hemorrhage data were recorded two ways. First, the total number of hemorrhage injuries observed for each individual rainbow trout was recorded. Second, as in past studies (e.g., Fredenberg 1992; Reynolds 1996), an overall hemorrhage rating was assigned to each fish, based on the largest individual hemorrhage injury observed.

Data analysis.—To assess the rate of healing, we evaluated total injuries present over time. Mortalities were not necropsied or included in the analysis because they died before the designated date of sacrifice, and any hemorrhages present did not have as much time to heal as surviving fish in the test lot. To compensate for unequal sample sizes resulting from mortalities and differences in original lot sizes, the mean number of injuries per necropsied fish was calculated for each sampling date. The rate of healing for any period was calculated using the equation: $H = 100 \times (N_{d1} - N_{d2})/N_{d1}$, where H = percent injuries healed, N_{d1} = mean

number of injuries per fish observed 1 d after exposure, N_{dt} = mean number of injuries per fish observed at time t.

Because the DC and PDC trials were conducted in different years and involved different fish sizes, temperatures, and power densities (Reynolds 1996), we did not attempt to statistically compare initial injury or healing rates for the two waveforms.

The proportion of fish in a test lot with one or more hemorrhage injuries was calculated for both shocked groups at each period. Binomial confidence limits were constructed for these proportions using the standard approximation formula of Zar (1974). The low injury rate observed in control samples for both DC and PDC trials did not allow for this approximation. Confidence limits in these instances were calculated using the more complex formula of Fleiss (1981) for binomial proportions approaching zero. Simple linear regression was used to separately assess the DC and PDC data for a relationship between time (days postshock) and mean injuries per necropsied fish. We compared observed slopes to zero to determine statistical significance (Zar 1974).

Results

Direct Current

We initially observed high numbers of hemorrhages and a rapid decline in incidence during the 5-week period. Only two control fish (4.1%) had hemorrhages, both level 1. Because this background rate was low, we did not adjust the treatment injury rates and thus present treatment injury rates with the knowledge that they contain a negligible number of hemorrhage injuries not related to electrofishing. In contrast to the low control injury rate, at 1 d postshocking, 86.1% of the fish exposed to DC had visible hemorrhages (Table 1). Hemorrhages per fish averaged 1.86, reaching a maximum of 5 injuries in one fish.

Initially, the majority of injuries were level 2. However, level-3 injuries observed per fish increased by 164% from day 1 to day 15 postshocking, whereas numbers of level-2 injuries declined by 43% during the same period (Figure 1). Thus, level-2 hemorrhages appeared to transition to level 3 during the initial 2 weeks postshocking. In addition, the amount of myomere tissue infused with blood in level-3 injuries increased substantially from 1 to 15 d postshocking. By day 15, coloration of all hemorrhages changed from bright red to duller shades of red and brown, with outside edges of many injuries growing faint.

By the end of the evaluation, there were marked reductions in all levels of injury in the DC test (Figure 1). The total number of injuries per fish began to decline markedly by 15 d postshocking. By the completion of the test at 36 d postshocking, there was a 78.0% reduction in total visible hemorrhages (Table 1). There was a highly significant ($P = 0.003$) negative relationship between injuries per fish in test lots and days postshock (Figure 2). Time explained 95% of the variation in injuries observed per fish (Figure 2). In all, 1.8% of the 502 trout exposed to DC died during the 36-d holding period. Water temperatures during the DC trial averaged 11.7°C.

Pulsed Direct Current

At 1 d postshocking, 81.6% of the fish exposed to PDC exhibited hemorrhages compared with only 1.9% of the control fish (1 of 53). Again, because of the low injury rate among controls, observed injury rates were not adjusted. Compared with DC, the PDC test group initially had a lower mean injury rate per fish, 1.45 at 1 d postshocking. As in the DC trial, we observed an increase (58.6%) in the number of level-3 injuries and a decrease in level-2 injuries (29.1%) 8 d after shocking, compared with the initial 1-d levels (Figure 1). However, healing occurred more rapidly compared with the DC trial; total injuries declined sharply at 15 d postshock (Table 1). By 57 d postexposure, a 92.4% reduction in the total visible hemorrhages per fish occurred. There was a highly significant ($P = 0.01$) negative relationship between injuries per fish and days postshocking; time explained 75% of the variation in injuries observed per fish (Figure 2). Examination of the graphic PDC data (Figure 2) suggests that the actual relationship between mean injuries per fish and time is not strictly linear and that healing rate slows with time. An exponential curve produced a stronger fit, explaining 93% of the variation ($y = 1.5e^{-0.0521x}$). Of the 708 rainbow trout exposed to PDC, 1.1% died during the 57-d holding period. Raceway water temperatures during the PDC trial averaged 14.5°C.

Spinal Injury

Evaluation of spinal injury healing was not a primary objective of this study. However, we observed spinal injuries including vertebral compressions and fractures during necropsies. Deposition of white connective tissue, fusing, and smoothing over fractured areas was readily apparent by 36 d in 1996 and 29 d in 1998. The

TABLE 1.—Injury and healing rates of electrofishing-induced hemorrhage injuries in hatchery rainbow trout exposed to DC and pulsed direct current (PDC); CI = confidence interval.

Sample and days postshock	Mortalities[a]	N	Number of fish by injury level[b]				Injury rate		Mean number injuries/ fish	Percent healed[c]
			0	1	2	3	Percent	95% CI		
DC										
Test										
1	0	101	14	10	56	21	86.1	7.0	1.86	—
8	2	98	6	6	51	35	93.9	4.0	1.83	1.6
15	3	97	10	4	30	53	89.7	5.4	1.42	23.7
22	1	99	33	3	29	34	66.7	9.4	0.86	53.8
36	3	98	63	2	18	15	35.7	9.7	0.41	78.0
Control										
1	0	49	48	2	0	0	4.1	2.8	0.02	—
PDC										
Test										
1	0	103	19	3	63	18	81.6	8.0	1.45	—
8	1	100	24	7	26	43	76.0	8.5	1.42	2.1
15	0	100	45	2	24	29	55.0	9.9	0.61	57.9
22	1	100	66	3	17	14	34.0	9.5	0.37	74.5
29	1	101	76	3	11	11	24.8	8.6	0.32	77.9
43	5	96	85	0	11	0	11.5	6.6	0.11	92.4
57	0	100	89	0	10	1	11.0	6.3	0.11	92.4
Control										
1	0	53	0	0	1	0	1.9	0.6	0.02	—

[a] Not used in analysis because any hemorrhage present did not have as much time to heal as survivors in a lot.
[b] Overall ranking based on the largest individual hemorrhage in each rainbow trout.
[c] Percent healed, calculated as the percent decline in mean injuries per fish from 1 d postshock.

smoothing and fusing of fractured areas was not observed 1 week before these dates in either the DC or PDC trial.

Discussion

To our knowledge, this is the first study that evaluates longevity and healing of electrofishing-induced hemorrhages in rainbow trout muscle. Hemorrhages occur through rupturing of blood vessels (Reynolds 1996), but our data suggest that healing begins soon after injury. The blood progressively diffused into adjacent muscle tissue, causing the injury to become enlarged, eventually dissipating. Although this observation is entirely descriptive and not based on the same individual fish, the process was readily apparent when observing the treatment lots temporally. Future studies should document the healing process with histological samples collected from injuries during each time period.

A purpose of this study was to document how long electrofishing hemorrhages persist in rainbow trout. For the two waveforms, we observed small (1.6–2.1%) declines in total number of injuries per fish by 8 d postshocking and a much larger decline (23.7–57.9%) by 15 d postshocking. Assuming the temporal difference in injuries observed per fish represents the healing rate, the majority of injuries healed by 22 d postshocking for both waveforms (Figure 2). Based on the 1996 results, we exposed two additional groups of fish to injury in 1998. This was to extend the observation period and hopefully document the healing of all injuries. By the end of 8 weeks (57 d) postshock, we classified 11.0% of the fish as injured (N = 100); 9 of the 11 injuries, however, appeared solely as light gray discolorations in the muscle bundles. Histological examination of specimens from three of these fish indicated that these areas were made up of breakdown products of red blood cells and macrophages mobilized to clean up necrotic tissue (K. Johnson, Idaho Department Fish and Game, personal communication). We believe the gray tissues were the last vestiges of the original injury. They were typically adjacent to obvious spinal injuries, which had also healed, as evidenced by fusion of two or more vertebrae. The gray tissues were quite faint, difficult to see, and did not resemble the classic red hemorrhages in myomeres that were typical of those observed in preceding weeks. We cannot conclusively define the time necessary for the complete healing of all injuries in the study, but our results suggest a likely time frame of 9–12 weeks.

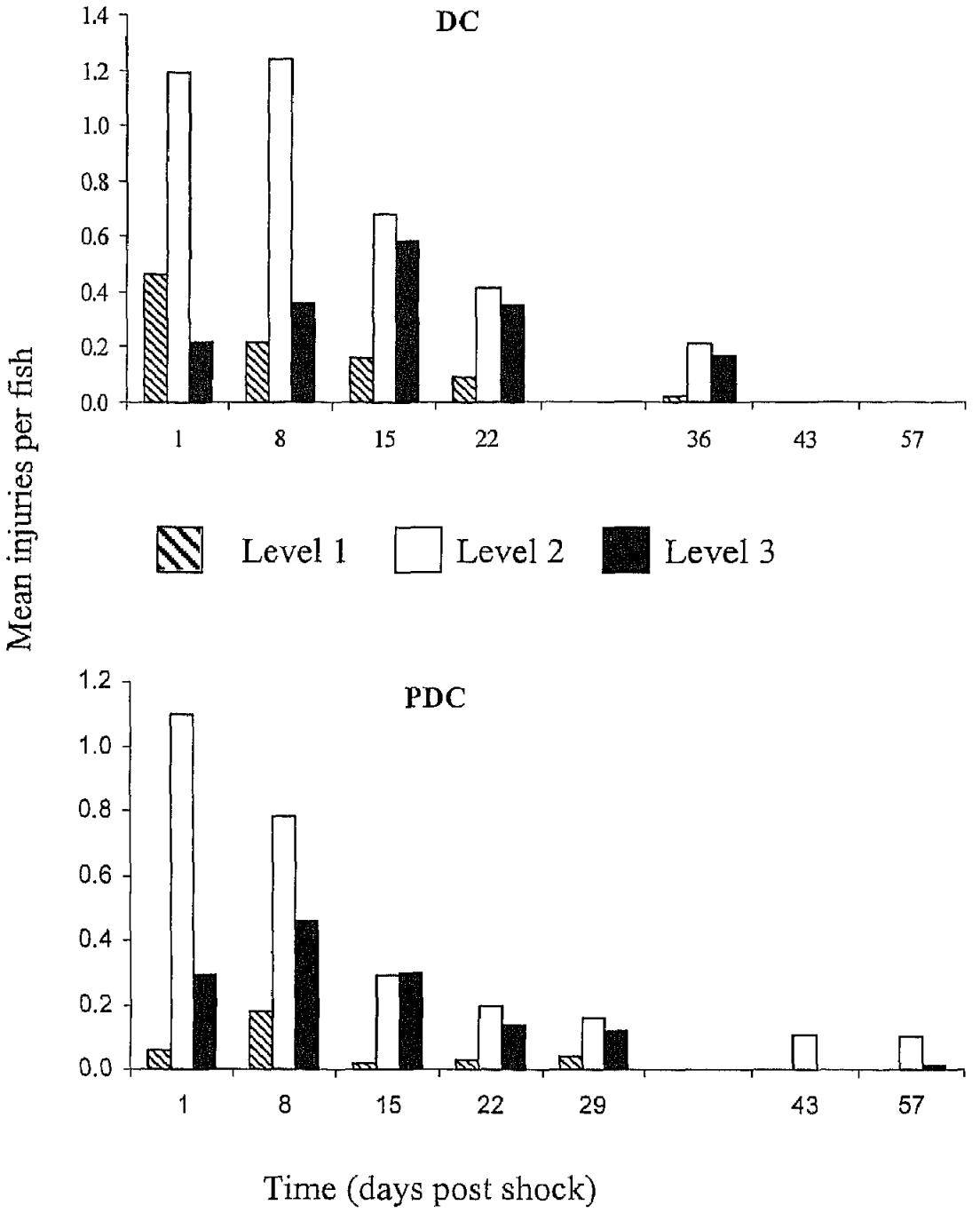

FIGURE 1.—The mean number of electrofishing induced hemorrhage injuries observed in hatchery rainbow trout exposed to DC or pulsed direct current (PDC) at various time intervals. Description of injury rating criteria (levels 1–3) is provided in the text.

There are several additional limitations to this study. These include the use of hatchery fish. Studies on wild rainbow trout might produce different healing rates. A similar study on wild rainbow trout would be problematic, however, requiring some type of sampling scheme involving large numbers of wild fish of similar length, sampled in their natural habitat, and held in an artificial environment, perhaps a pond (e.g., Dalbey et al. 1996) or artificial stream channel that can be drained without resampling via electrofishing or seining. Because wound repair can be delayed or inhibited in fish on altered diets (Halver et al. 1969), such an approach could also yield artificial results. Further, it seems reasonable to suspect that different temperatures might result in faster or slower repair of blood vessel injuries. The trials conducted in this study had modestly different mean temperatures, but differences in waveforms and power density exposures precluded evaluation of temperature effects.

If future researchers evaluate hemorrhage healing rates over time they will be faced with a two-dimensional versus three-dimensional classifica-

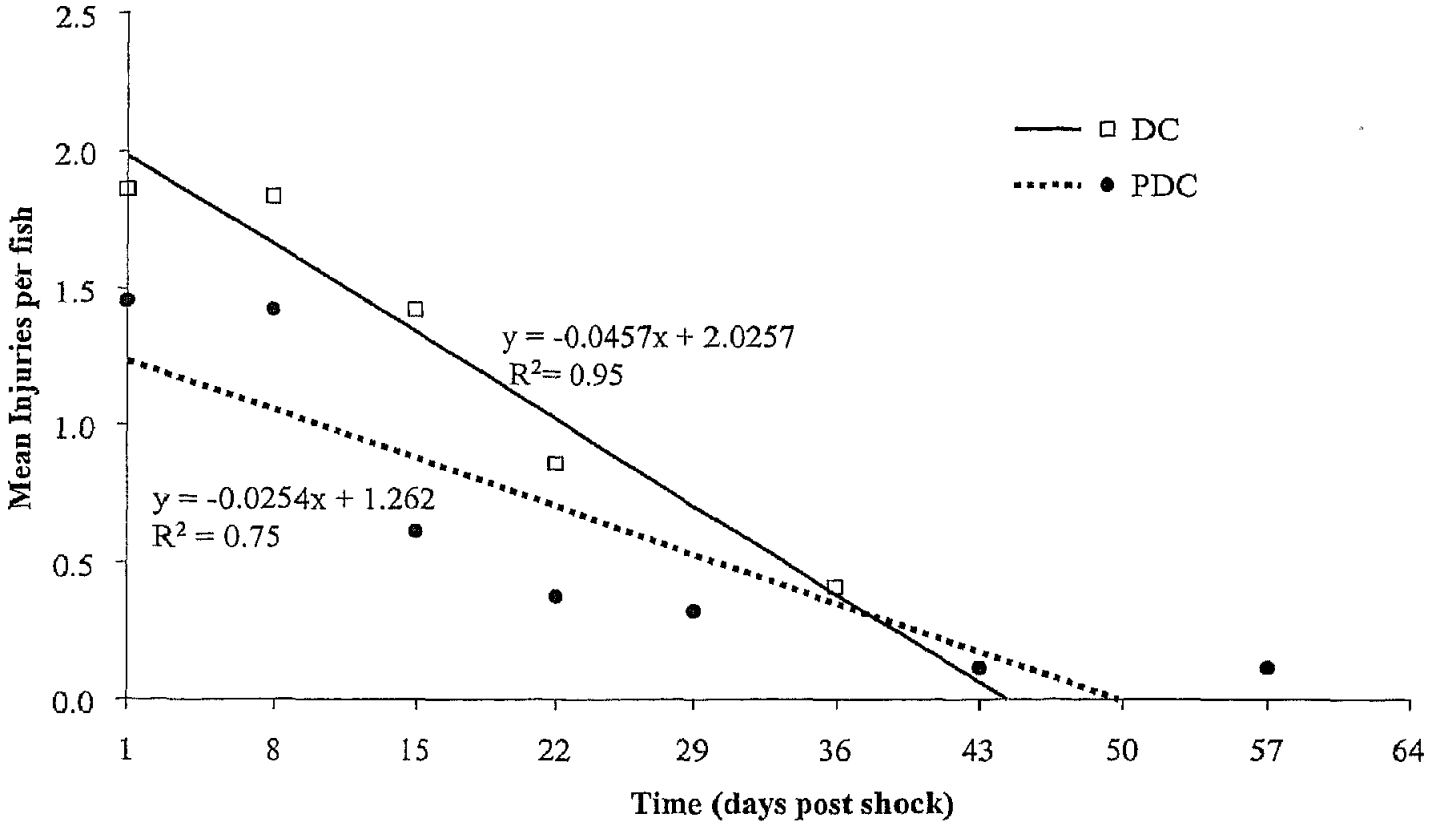

FIGURE 2.—Simple linear regressions of time (days postshock) versus injuries observed per necropsied hatchery rainbow trout shocked with DC or pulsed direct current (PDC).

tion dilemma. Based on our classifications, the number of level-3 injuries increased during the initial 2–3 weeks and then declined. We believe this occurred as a result of level-2 injuries spreading to level-3 injuries as the blood diffused outward into the myomeres away from the spinal column following initial blood vessel ruptures. In these cases, the hemorrhages began at a vertebrae and sometimes became quite long, often well above the length criteria for a level-3 injury (wounds >2 vertebrae widths). However, much of this elongation did not occur *on the spine* (the other criteria for a level-3 injury). Rather, injury elongation occurred from near the spine outward along individual muscle fibers towards the skin. Because there was no precedent for this rating dilemma, we opted to rate such injuries as level 3.

The change in injury ratings over time suggests that subjectivity is an important limitation of the 0–3 hemorrhage rating scale currently employed. Given the scale descriptions, we could easily have placed some injuries into several categories, even when completing necropsies on trout 1 d postshocking. For example, many of the hemorrhages we classified as level 2 were band-like in nature, extending from myomeres up or down to the edge of the spine. Such injuries would have been classified as level 1 by another researcher because the injury did not occur directly on the spine (J. Reynolds, Alaska Cooperative Fish and Wildlife Research Unit, personal communication).

Arbitrariness is an additional limitation with the current hemorrhage rating scheme. Reynolds (1996) noted these ratings are not linear measurements, and their actual relation to fish health has not been established. In fact, nothing is known about the appropriateness of the 0–3 scale because after the fish has been necropsied, it is impossible to associate various levels of hemorrhage injuries with subsequent levels of mortality or growth (Thompson et al. 1997). Although the limitations of the scale are apparent to anyone who has used it, the rapid healing of most injuries may render these concerns moot.

Hemorrhage injury rates in our tests are higher than rates reported in most field studies (Sharber and Carothers 1988; Holmes et al. 1990; Fredenberg 1992; McMichael 1993; Hollender and Carline 1994). Typically, field conditions result in most fish being exposed to increasing electrical stimulus as they enter the electrical field and moving towards the electrode. In our study all fish were instantaneously exposed to high field intensity in a small dip net. The instant exposure to high field intensity may have caused the higher levels of injury (Reynolds, personal communication). To con-

duct this study it was necessary to induce high levels of hemorrhage injury to facilitate subsequent observations of healing. Thus our overall injury levels should not be considered reflective of field conditions.

If hemorrhage injuries are short term and do not represent a long-term detrimental impact to the fish, biologists may need to reevaluate criteria for reporting hemorrhage injuries related to electrofishing. Based on a review of several studies (Holmes et al. 1990; Fredenberg 1992; McMichael 1993; Hollender and Carline 1994), it appears that hemorrhage injuries constitute about half of the total injuries being reported. The practice of combining hemorrhage and spinal injury ratings, as has often been done in the past, assumes such injuries have equivalent impacts on growth and survival of the injured fish, an unlikely proposition. Because of their apparent ephemeral nature in hatchery rainbow trout, as reported in this study, we suggest hemorrhages be reported and analyzed separately from spinal injuries in future studies.

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