# The Effects of Electroshock on Immune Function and Disease Progression in Juvenile Spring Chinook Salmon

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*Abstract.*—Although much is known about the effects of electroshock on fish physiology, consequences to the immune system and disease progression have not received attention. Our objectives were to determine the effects of electroshock on selected immune function in juvenile spring chinook salmon *Oncorhynchus tshawytscha,* the mechanism of any observed alteration, and the effects of electroshock on disease progression. We found that the ability of anterior kidney leukocytes to generate antibody-producing cells (APC) was suppressed 3 h after a pulsed-DC electroshock (300 V, 50 Hz, 8 ms pulse width) but recovered within 24 h. This response was similar in timing and magnitude to that of fish subjected to an acute handling stress. The mechanism of suppression is hypothesized to be via an elevation of plasma cortisol concentrations in response to stress. Other monitored immune functions, skin mucous lysozyme levels, and respiratory burst activity were not affected by exposure to electroshock. The progression of a *Renibacterium salmoninarum* (RS) infection may have been altered after exposure to an electroshock. The electroshock did not affect infection severity or the number of mortalities, but may have accelerated the time to death. The limited duration of APC suppression and lack of effects on lysozyme and respiratory burst, as well as infection severity and mortality levels in RS-infected fish, led us to conclude that electrofishing under the conditions we tested is a safe procedure in regards to immunity and disease.

The immediate physiological effects of electroshock on fish are well documented. Overt physical damage resulting from exposure to AC or DC can include spinal injury, hemorrhages, and blood clots (Hauck 1949; Sharber and Carothers 1988; Hollender and Carline 1994; Sharber et al. 1994). Mortality can also result directly from exposure to current or indirectly as a result of physical damage

(Hauck 1949; Hudy 1985). Physiological changes reported in fish exposed to electroshock include elevated levels of plasma cortisol catecholamine, lactic acid, and glucose (Schreck et al. 1976; Mesa and Schreck 1989; Maule and Mesa 1994; Mitton and McDonald 1994; Barton and Grosh 1996). Increased levels of these compounds are all indicative of a stress response in fish (Mazeaud et al. 1977).

Mortality and injury rates can vary according to many factors, including the type of current used. Rates of mortality and injury are highest with AC, intermediate with pulsed DC, and lowest with DC (Lamarque 1990). Mortality of fingerling chinook salmon *Oncorhynchus tshawytscha* exposed to pulsed DC rises with increasing exposure duration, pulse frequency, and water temperature (Collins et

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al. 1954). Increased injury rates in rainbow trout *O. mykiss* have been related to higher pulse frequencies (McMichael 1993; Sharber et al. 1994). The frequency and severity of injuries may also vary by species (McMichael 1993; Sharber et al. 1994; Bardygula-Nonn et al. 1995; Sharber and Carothers 1988). Exposure to electroshock may reduce growth for periods of a month in rainbow trout, arctic grayling *Thymallus arcticus,* and Yellowstone cutthroat trout *O. clarki bouvieri* (Dwyer and White 1995) up to a year in rainbow trout and brown trout *Salmo trutta* (Gatz et al. 1986). Electroshock has also been shown to alter behavior and feeding rates in cutthroat trout *O. clarki* (Mesa and Schreck 1989).

The effects of exposure to electroshock on immune function and disease resistance in fish are not known. Exposing fish to an acute electroshock, however, does elicit a stress response that probably provokes, at least temporarily, altered responses of certain immune functions. An acute stress temporarily reduces antibody-producing cell production and disease resistance in juvenile chinook salmon (Maule et al. 1989). Other immune factors are also altered by stress. Lysozymes are a group of enzymes found in skin mucus, most tissues, and plasma that contribute to immunity by destroying cell walls of many types of bacteria (Alexander and Ingram 1992). Plasma lysozyme activity in rainbow trout increased after a handling stress of 30 min or less (Möck and Peters 1990; Demers and Bayne 1997) but was reduced after a 2-h transportation stress (Möck and Peters 1990). Phagocyte respiratory burst activity, a process by which oxygen free radicals that kill foreign cells are produced (Secombes and Fletcher 1992), is reduced after stress in Atlantic salmon *Salmo salar* (Thompson et al. 1993) and rainbow trout (Angelidis et al. 1987).

We had three research objectives: (1) to determine the effects of electroshock on the responses of selected immune functions in juvenile spring chinook salmon and to place any change in the context of an acute handling stress—a stressor known to alter these responses, (2) to determine the mechanism of any alteration (i.e., separate possible effects at the organism level from direct effects on leukocytes) by monitoring the stress response of electroshocked fish and the responses of immune cells exposed to an electroshock in vitro, and (3) to determine the effects of electroshock on disease progression and again place any change in the context of an acute handling stress—a stressor known to alter disease resistance.

## Methods

*Experimental animals*.—Juvenile spring chinook salmon were obtained from the Fish Performance and Genetics Laboratory at Smith Farm (SF; Oregon State University, Corvallis) or the Little White Salmon National Fish Hatchery, Washington. Disease challenges were conducted at the Salmon Disease Laboratory (SDL; Oregon State University, Corvallis) and at the Columbia River Research Laboratory, Cook, Washington (CRRL). Immune function experiments were conducted at CRRL and SF. Fish for experiments conducted at SF were maintained in circular fiberglass tanks (61-cm diameters, water depths of 45 cm) and at SDL in 100-L cuboidal fiberglass tanks (water depths of 27 cm), all provided with flow-through well-water (12°C, ambient conductivity of 530–533 μS/cm). Fish for experiments conducted at CRRL were maintained in circular fiberglass tanks (diameters and water depths: 61 and 45 cm for immune function experiments and 86 and 71 cm for disease challenge experiments) provided with flow-through well-water heated to 12°C, (ambient conductivity 66–71 μS/cm). Fish at all locations were fed a commercial moist pellet feed offered daily to satiation.

*Immune function experiments*.—Immune competence after electroshock was assessed in experiments conducted in August 1996 at CRRL (immune experiment 1 or $IE_1$), September 1996 at SF (immune experiment 2 or $IE_2$), and August 1997 at CRRL (immune experiment 3 or $IE_3$). Fish were stocked randomly at 20 fish/tank for $IE_1$ (mean fork length and weight = 107 mm and 13.8 g) and 25 fish per tank for $IE_2$ (100 mm and 12.2 g) and $IE_3$ (167 mm and 50.6 g). Tanks were assigned treatments by random drawing. Following a 2-week acclimation period, fish in replicate tanks were treated one of three ways: (1) a pulsed DC electroshock (300 V, 50 Hz, 8 ms pulse width) for 2 s from a Smith-Root (POW 12b) backpack electrofisher, (2) a handling stress, in which fish were capture by dip net and suspended out of water for 30 s, or (3) no treatment (control). Fish were sampled at 3 h, 24 h, and 7 d after treatment. At each sample time four ($IE_1$) or five ($IE_2$ and $IE_3$) fish were rapidly netted from each tank and transferred into a lethal dose of anesthesia (buffered MS-222, 200 mg/L). After fish were fully anesthetized, fork lengths and weights were measured.

In $IE_1$ we monitored lysozyme levels in skin mucus as an indicator of nonspecific immunity. A sample was collected from each fish with a 10-

μL-inoculation loop wiped across an area near the lateral line above the vent. The sample was removed with the loop tip perpendicular to the fish, to ensure only the aperture was coated, and placed in 100 μL of 0.04 M phosphate buffer. Samples were thoroughly mixed with buffer and stored at $-80°C$. We determined lysozyme levels with a microplate assay developed from the methods of Litwack (1955) as modified by Sankaran and Gurnanai (1972) following Muona and Soivio (1992). Briefly, 25 μL of each mucus and buffer mixture was combined with 250 μL of 0.25 g/L *Micrococcus lysodeikticus* in a 0.02-M acetate buffer suspension in the wells of a 96-well microplate and incubated at $37°C$. The absorbances were measured with a spectrophotometer at 450 nm at time 0 and again after 20 min. A standard curve was established using hen egg-white lysozyme. The lysozyme activity in a sample (mg/μL hen egg-white lysozyme) is proportional to the decrease in absorbance over time.

We measured plasma cortisol and glucose levels to monitor the stress response of fish. The caudal peduncle was severed and blood was collected in heparin-coated capillary tubes. Plasma was separated from whole blood by centrifugation; samples were initially frozen at $-20°C$ and stored at $-80°C$. Plasma cortisol concentrations were determined by radioimmunoassay (Redding et al. 1984). Plasma glucose concentrations were determined with a glucose-hexokinase assay (Sigma Procedure 16-UV, Sigma Diagnostics, St. Louis, Missouri).

Anterior kidney leukocytes were isolated to assess their ability to form specific antibody-producing cells (APC). The anterior kidney was aseptically harvested and placed in ice-cold tissue culture medium (TCM) consisting of RPMI 1640 supplemented with 10% fetal calf serum, 1% L-glutamine, and 0.1% gentamicin. A leukocyte suspension was made by using the plunger of a 1-mL tuberculin syringe to gently push tissue and media through a 100-μm mesh onto a sterile petri dish. The mesh was washed with fresh TCM, and the suspension was aspirated and placed on ice in a 5-mL, sterile capped tube. The suspension was centrifuged for 10 min at $500 \times$ gravity ($4°C$). The supernatant was aspirated and discarded, cells were then resuspended in 0.5 or 1.0 mL of fresh TCM. Viable leukocytes were counted, and each suspension was diluted to a working concentration of $2 \times 10^7$ cells/mL using the methods of Slater et al. (1995). Duplicate 50-μL aliquots ($10^6$ cells) of each suspension were placed in wells of a 96-

well microplate along with 50 μL TCM containing the antigen trinitrophenol-lipopolysaccharide (TNP-LPS). Production of antibody-producing cells was assessed in vitro by using a slightly modified hemolytic plaque assay (Slater et al. 1995). The only modification was the use of diluted (1:10) rainbow trout serum as the complement source, rather than steelhead *O. mykiss* serum.

Phagocytes were isolated to measure their respiratory burst activity using a modification of the methods of Field et al. (1995). Briefly, after aliquots for the plaque assay were removed, the working cell suspension was centrifuged for 10 min at $500 \times$ gravity ($4°C$) and the supernatant was aspirated and discarded. The cells were then resuspended in ice-cold TCM consisting of RPMI 1640 supplemented with 0.1% fetal calf serum, 1% L-glutamine, and 0.1% gentamicin (0.1% TCM) to the original volume of the working concentration minus the aliquots removed for quantifying APC. Triplicate 100-μL aliquots of each suspension were placed in wells of a 96-well microplate, covered, and incubated for 1 h at $17°C$ to allow phagocytes to adhere. Cells were washed twice with 0.1% TCM to remove nonadherent cells. Respiratory burst activity was measured with Secombes' (1990) nitroblue tetrazolium (NBT) assay for detection of intracellular superoxide anion ($O_2^-$). We incubated cells with the NBT solution for 2 h at $17°C$. Absorbances were measured with a spectrophotometer at 630 nm after the plate was shaken for 60 s.

In $IE_2$, we measured only plasma glucose and cortisol concentrations and APC production. Cortisol and glucose levels were determined as in $IE_1$, whereas APC production was assessed following the methods of Slater et al. (1995). The methods for $IE_3$ were the same as for $IE_1$, except for APC production, which was assessed using the methods of Slater et al. (1995).

*In vitro experiments.*—We investigated the direct effects of electroshock on leukocyte responses in two in vitro experiments ($VE_1$ and $VE_2$) in September 1997 at CRRL. Ten fish (mean fork length 171 mm; mean weight 58.7 g) for each in vitro experiment were rapidly netted and transferred into a lethal dose of anesthesia. Anterior kidney leukocytes were harvested, suspended in TCM, and centrifuged as described for $IE_1$. The supernatant was aspirated and discarded, and cells were resuspended in 2 mL TCM. Duplicate 1-mL aliquots of each suspension were placed in short lengths of dialysis tubing (molecular weight cutoff: 6,000–8,000) that had been soaked and rinsed

with distilled-deionized water, rinsed with TCM, and sealed at one end with a weighted plastic clip. After the suspension was loaded in the tubing, the open end was folded down twice, sealed, and placed on ice. One suspension from each fish was randomly assigned for treatment and the other as control; thus, each fish acted as its own control.

Five loaded sections of tubing at a time were suspended with equally spaced lengths of string from a piece of PVC pipe. Each sample was suspended just under the surface of the water in a 61-cm diameter circular fiberglass tank filled with 9°C well-water (ambient conductivity 70 μS/cm). Electrodes were placed on opposite sides of the tank, and the length of pipe was positioned across the tank so that current flow would be perpendicular to the pipe and the samples midway between the electrodes. Treatment suspensions were exposed to a pulsed DC electroshock as in $IE_1$, removed from the tank, and immediately placed on ice. Control suspensions were treated identically but without the electroshock. The tubing of each sample was cut just above the fluid; the suspension was aspirated and placed on ice in a 5-mL, sterile capped tube. Some fresh TCM was added to the tubing to remove cells, aspirated, and added to the suspension. Suspensions were centrifuged for 10 min at $500 \times$ gravity (4°C), the supernatant was aspirated and discarded, and cells were resuspended in 0.5 mL TCM. Leukocytes were quantified and diluted then assessed for APC production and respiratory burst activity as in $IE_1$.

*Disease challenges.*—Effect of electroshock on disease progression in juvenile chinook salmon was tested in January 1997 at SDL (disease challenge 1 or $CH_1$) and August 1997 at CRRL (disease challenge 2 or $CH_2$). In $CH_1$, fish (mean fork length 135 mm; mean weight 28.4 g) with a natural infection of *Renibacterium salmoninarum,* the pathogenic cause of bacterial kidney disease, were stocked and allowed to acclimate for at least 2 weeks. We identified the pathogen by a combination of low-level mortality and gross pathology with confirmation by laboratory analysis as described below. Fish were stocked from the same source, so we assumed that all had been exposed to *R. salmoninarum* (hereafter referred to as RS) and that incidence of infection was initially equal in all tanks.

Following acclimation, treatments were assigned to replicate tanks (3 tanks/treatment and 30 fish/tank) by random drawing. Fish were treated with an electroshock, a handling stress, or no treatment (control) as described for $IE_1$. Control tanks were divided among three sample times: weeks 0 (start of the experiment), 2, and 4. Electroshock and handling-stress tanks were sampled at either 2 or 4 weeks posttreatment. Dead fish were removed daily and tallied. At each sample time all fish from a tank were rapidly netted and transferred into a lethal dose of anesthesia. We recorded the fork length and weight of each fish and then collected and pooled kidney and spleen tissues. Tissues were collected using methods to avoid cross-contamination between individuals (Pascho et al. 1987). Samples were stored at −80°C. We confirmed infection levels of and mortality due to RS with an enzyme-linked immunosorbent assay (ELISA; Pascho et al. 1991). Analysis by ELISA yields optical density (OD) values, which translate to severity of infection (OD values $<0.2$ = low-level infections, 0.2–1.0 = moderate, and $>1.0$ = high).

Fish for $CH_2$ (mean fork length 109 mm; mean weight 18.4 g) were stocked at 130 fish/tank and allowed to acclimate for 2 weeks. After acclimation, each of four treatments was assigned to duplicate tanks by random drawing. Seven fish from each tank were sampled, as in $CH_1$, to determine baseline RS levels. Fish were then exposed to RS via a modified waterborne immersion challenge (Mesa et al. 2000). Alterations from this method included an 8-d incubation of the bacteria, tank water volumes of 75 L during the challenge, and a challenge with 75 mL of bacteria–saline suspension (treatment and positive control tanks) or a sham challenge with 75 mL of sterile peptone saline (negative control tanks). The concentration of RS in the bacteria–saline suspension was determined to be $9.5 \times 10^8$ bacteria/mL for an immersion challenge concentration of $9.5 \times 10^5$ bacteria/mL. Every 2 weeks, 7 fish from each tank were sampled, as in $CH_1$, to monitor RS progression. Seven weeks after immersion challenge, fish were treated with an electroshock, handling stress, or no treatment (control), as in $IE_1$. Five fish from each tank were sampled at 3 h, 24 h, and 72 h poststress to monitor physiological responses to each treatment. Blood was collected and plasma isolated, stored, and analyzed for cortisol and glucose concentrations, as described in $IE_1$. Disease progression was monitored 4 d after the stress treatments (8 weeks after immersion challenge) and continued biweekly for another 10 weeks.

*Data analyses.*—Replicate tanks from all immune experiments were compared by two-way *t*-tests, with the exception of APC data and $IE_3$ lysozyme data, where we used the nonparametric

Wilcoxon's rank-sum test. Triplicate tanks from $CH_1$ were compared by general linear models (GLM) $F$-test (SAS Institute 1994). In $CH_2$, replicates of cortisol, glucose, and mean time to death were compared by two-way $t$-tests and mean OD data by Wilcoxon's rank-sum test. Replicates not found to be different ($P > 0.05$) were pooled for further analysis. Box-plots and residual plots were constructed to identify outliers and to examine relative spreads for identifying data in need of transformation. We multiplied NBT values by 100 before transformation to avoid negative values that result when $\log_e$-transforming values between 0 and 1 (Sokal and Rohlf 1981). We analyzed all immune experiment data by GLM $F$-test, except for APC and $IE_3$ lysozyme data, where we used the nonparametric Kruskal–Wallis test. All multiple comparisons were made using the least-significant-difference (LSD) test. Nonparametric tests were used on data with numerous outliers and in $IE_3$ (due to several fish with no detectable lysozyme). We analyzed in vitro experiment data by paired $t$-test. Disease challenge data were analyzed by GLM $F$-test ($CH_1$) or Kruskal–Wallis test ($CH_2$) and multiple comparisons by LSD test.

## Results

### Immune Function Experiments

Data from replicates in all immune experiments did not differ, so they were pooled for further analysis. Skin mucous lysozyme concentrations did not differ between electroshocked and control fish at any time in $IE_1$ and $IE_3$ (Table 1). Respiratory burst activity did not differ by treatment in $IE_1$ but did in $IE_3$ ($\log_e$-transformed data, $P = 0.027$). Activity was greater in electroshocked fish compared with control and handled fish 7 d after stress ($P \leq 0.035$; Table 1); activity in controls at 7 d was also significantly greater than at 3 h ($P = 0.005$).

In $IE_1$ APC generation was significantly lower in electroshocked fish compared with control or handled groups 3 h after stress ($P \leq 0.014$) but returned to control levels within 24 h (Table 1). Control levels at 3 h and 24 h in $IE_1$ were 74 and 18 APC/$10^6$ leukocytes, respectively. In $IE_2$, handled fish had significantly fewer APC than did controls 3 h after stress ($P = 0.007$). Electroshocked fish also had fewer APC than controls at 3 h, but the difference was not statistically significant ($P = 0.06$). Treatments did not differ at 24 h or 7 d after stress. Control levels at 3 h, 24 h, and 7 d in e $IE_2$ were 683, 816, and 384 APC/$10^6$ leukocytes, respectively. In $IE_3$, APC production did not differ

by treatment at any time (Table 1). Control levels at 3 h, 24 h, and 7 d in $IE_3$ were 75, 168, and 229 APC/$10^6$ leukocytes, respectively.

Plasma cortisol levels varied by treatment in $IE_1$ and $IE_3$ ($\log_e$-transformed data) and $IE_3$ (square-root-transformed data; $P \leq 0.002$). In $IE_1$, cortisol levels in electroshocked and handled fish were significantly greater than in controls at 3 h after stress in all three immune experiments ($P \leq 0.009$; Table 1) and returned to control levels within 24 h. The electroshock and control groups did not differ at 7 d in $IE_1$ and $IE_2$. In $IE_3$, levels among controls at 7 d were significantly greater than for the other treatments ($P \leq 0.03$). Levels in controls at 7 d were also significantly greater at 24 h ($P = 0.008$; Table 1), whereas the electroshock and handled groups did not differ at 7 d or 24 h.

Plasma glucose levels differed by treatment in $IE_1$ ($\log_e$-transformed data) and $IE_3$ ($P = 0.0001$), but not in $IE_2$. In $IE_1$, glucose concentrations in electroshocked fish at 3 and 24 h poststress were significantly greater than in control and handled fish ($P \leq 0.029$), but differences were not significant at 7 d. Glucose concentrations in handled fish were also significantly greater than in controls at 3 h ($P = 0.0008$) but returned to control levels within 24 h (Table 1). In $IE_3$, glucose levels in both electroshock and handled groups were significantly greater than in controls 3 h after stress ($P \leq 0.0003$). Concentrations in electroshocked fish returned to control levels within 24 h but remained elevated above the other treatments in handled fish ($P \leq 0.008$). Glucose levels did not vary by treatment at 7 d.

### In Vitro Experiments

Respiratory burst activity did not differ between electroshocked and control leukocyte suspensions during $VE_1$ (data not shown). Activity in the electroshock group was significantly greater than in controls during $VE_2$ ($P = 0.0057$; Figure 1). Production of APC by leukocytes did not differ by treatment during $VE_1$ or $VE_2$ (data not shown).

### Disease Challenges

In $CH_1$, with one exception, no differences were detected in triplicate tanks. Data from replicates that did not differ were pooled for further analysis. One tank of handled fish sampled 2 weeks poststress differed significantly from two other replicates ($P = 0.0001$) and was included in the analysis as a separate treatment. Mean OD values varied by treatment in $CH_1$ ($P = 0.0001$). Mean OD values of controls did not differ throughout the

TABLE 1.—Physiological and immune indicators measured in immune experiments 1, 2, and 3 in juvenile spring chinook salmon three times (3 h, 24 h, 7 d) after treatment with either a 2-s, 300-V, 50-Hz, 8-ms-pulse-width pulsed-DC electroshock (electroshock); a 30-s handling stress (handling); or no stress (control). Values are means (SE) that include data from two replicates that did not differ significantly and were pooled ($N$ = 8–10). Means within a sample time without a letter in common differ significantly; values within a treatment across sample times marked with an asterisk differ significantly.

| Factor measured[a] | Treatment | Immune experiment 1 | | | Immune experiment 2 | | |
|---|---|---|---|---|---|---|---|
| | | 3 h | 24 h | 7 d | 3 h | 24 h | 7 d |
| Lysozyme | Control | 9.3 (2.5) | 9.1 (1.5) | 7.9 (2.3) | | | |
| | Electroshock | 9.1 (2.9) | 7.7 (1.5) | 11.5 (3.7) | | | |
| | Handling | 11.9 (3.5) | 9.1 (1.3) | 12.6 (1.8) | | | |
| Respiratory burst | Control | 0.830 (0.10) | 0.401 (0.07) | 0.332 (0.07) | | | |
| | Electroshock | 0.964 (0.27) | 0.857 (0.18) | 0.151 (0.03) | | | |
| | Handling | 0.723 (0.13) | 0.559 (0.10) | 0.299 (0.05) | | | |
| APC | Control | 100.0 (43.0) z | 100.0 (13.6) | | 100.0 (23.2) z | 100.0 (21.6) | 100.0 (29.2) |
| | Electroshock | 14.6 (3.7) y | 88.7 (16.1) | | 50.1 (18.3) y | 90.5 (23.6) | 112.5 (32.8) |
| | Handling | 43.5 (11.0) z | 141.2 (27.1) | | 25.2 (11.7) y | 50.5 (14.5) | 123.3 (27.3) |
| Cortisol | | | | | | | |
| | Control | 2.2 (0.9) z | 4.2 (1.6) | 2.5 (0.7) | 11.9 (6.2) z | 3.0 (0.7) | 5.3 (1.3) z |
| | Electroshock | 24.9 (7.6) y | 8.5 (4.6) | 11.8 (5.4) | 121.2 (22.6) y | 14.1 (3.7) | 12.4 (4.1) z |
| | Handling | 9.0 (1.4) y | 8.0 (2.8) | 7.4 (2.7) | 57.9 (23.0) y | 8.8 (3.6) | 55.8 (24.2) y |
| Glucose | Control | 67.6 (2.8) z | 62.6 (2.6) z | 62.1 (3.0) | 60.8 (2.7) | 79.8 (4.1) | 89.4 (8.1) |
| | Electroshock | 123.5 (11.3) y | 88.0 (4.8) y | 57.5 (1.7) | 75.5 (9.1) | 86.0 (17.4) | 90.1 (5.1) |
| | Handling | 95.2 (6.8) x | 66.6 (1.8) z | 64.8 (2.5) | 77.8 (5.9) | 82.9 (15.3) | 79.1 (8.7) |

[a] Skin mucous lysozyme levels are reported as g/mL hen egg-white lysozyme, respiratory burst activity as nmoles nitroblue tetrazolium-reduced/$10^6$ leukocytes, antibody producing cell generation (APC) as percent of controls, cortisol levels as ng/ml plasma, and glucose concentrations as mg/dL plasma.

challenge. The mean OD of electroshocked fish at 2 weeks was significantly greater than in controls and handled fish but lower than one tank of handled fish ($P \leq 0.027$; data not shown). The week-2 electroshocked fish did not differ from baseline (week 0) controls. The week-4 electroshock group did not differ from controls at week 0 or week 4 (data not shown).

In $CH_2$ no differences in mean OD were found in replicates within positive controls or either treatment, so these data were pooled for further analysis. One negative control tank was removed from the analysis because it became infected with RS. The source of infection is unknown, although cross-contamination from an RS-treated tank seems likely because only one negative control tank was affected. The infection produced low mortality, which began approximately 12 weeks after the start of the experiment, and significantly different mean OD values between the negative control tanks at four of nine sample times ($P \leq 0.05$).

Before challenge with RS mean ELISA ODs did not differ by treatment (Figure 2). Mean ELISA ODs differed by treatment from weeks 2 through 12 after infection with RS ($P < 0.0002$). All RS-challenged groups had significantly greater mean ODs than the negative controls over the same period ($P < 0.004$). Mean ODs increased in all RS-challenged groups through week 6, positive controls being significantly lower than both the electroshock and handled groups at 2 and 4 weeks after challenge ($P < 0.007$; Figure 2). The mean ODs of the RS-challenged groups did not differ at 6 weeks. At 8 weeks after challenge the electroshock and handled group's mean ODs did not differ, but they were significantly greater than in the positive controls ($P \leq 0.045$). No differences were detected between RS treatments 10 weeks after challenge and all had mean ODs over 1.0, the cutoff for high-level infections. The mean OD of the positive control group 12 weeks after challenge was significantly greater than in the electroshock and handled groups ($P \leq 0.032$), but these two treatments did not differ. Mean ODs did not differ by treatment at 14, 16, or 18 weeks after challenge.

Neither plasma cortisol or glucose concentrations varied by replicate in $CH_2$, so data were pooled for further analysis. Plasma cortisol levels varied within RS treatments at 3 h and 24 h after stress ($\log_e$-transformed data, $P \leq 0.02$); at 3 h after treatment, levels in electroshocked and handled fish were significantly greater than in positive controls ($P \leq 0.0005$; Table 2). The levels in electroshocked fish were still greater than in the positive controls at 24 h ($P = 0.028$), but treatments did not differ from controls at 72 h after stress. Plasma glucose levels differed by treatment at 3 h

TABLE 1.—Extended.

| Factor measured | Immune experiment 3 | | |
|---|---|---|---|
| | 3 h | 24 h | 7 d |
| Lysozyme | 4.2 (2.6) | 13.4 (3.9) | 24.1 (5.1) z |
| | 7.4 (3.2) | 15.3 (3.7) | 16.7 (5.4) z |
| | 1.9 (1.3) | 13.4 (3.2) | 5.6 (2.8) y |
| Respiratory burst | 0.334 (0.05)* | 0.495 (0.06) | 0.945 (0.28) z |
| | 0.342 (0.04) | 0.398 (0.03) | 1.450 (0.26) y |
| | 0.388 (0.09) | 0.678 (0.20) | 0.728 (0.11) z |
| APC | 100.0 (23.2) | 100.0 (22.9) | 100.0 (6.2) |
| | 88.7 (26.1) | 72.3 (25.2) | 60.2 (12.7) |
| | 84.8 (25.4) | 59.1 (15.0) | 100.2 (20.3) |
| Cortisol | 24.6 (8.7) z | 14.5 (5.2)* | 42.8 (11.4) z* |
| | 60.3 (8.9) y | 8.8 (2.5) | 11.4 (1.8) y |
| | 53.0 (7.1) y | 14.9 (4.6) | 16.7 (6.1) y |
| Glucose | 79.6 (4.5) z | 89.2 (3.5) z | 88.0 (7.7) |
| | 110.7 (6.0) y | 99.2 (9.5) z | 71.7 (3.4) |
| | 113.9 (5.9) y | 121.7 (5.9) y | 81.1 (2.8) |



FIGURE 1.—Difference in respiratory burst activity (nmol NBT reduced/$10^6$ leukocytes) between control and treatment head kidney leukocyte suspensions from juvenile spring chinook salmon. Treatment consisted of an in vitro 2-s, 300-V, 50-Hz pulsed DC electroshock. Each fish from in vitro experiment 2 ($N = 10$) served as its own control; thus, each point represents the response of control versus treatment leukocyte suspensions from an individual fish. The diagonal represents equal responses.

after stress ($P = 0.0003$). Levels for electroshocked and handled fish were significantly greater than for positive controls at 3 h after treatment ($P \leq 0.004$). No differences were detected between RS treatments at 24 h or 72 h after stress.

The number of mortalities varied within and among treatments (Figure 3). Because samples were collected from each tank at 2-week intervals, we could not determine the percentage mortality. Mean time to death did not differ in duplicate tanks for any treatment, so the data were pooled for further analysis. Compared with the positive controls, the mean time to death was 5 d earlier for electroshocked fish and 2.5 d earlier for handled fish. Evidence that mean time to death was significantly affected by treatment was inconclusive ($P = 0.08$). Multiple comparisons based on this analysis indicated the difference between electroshocked and positive control fish was significant ($P = 0.025$).

## Discussion

Although the impacts of electrofishing have been thoroughly investigaed, our study is the first to examine the effects of electroshock on immune function. We found that a single electroshock of juvenile spring chinook salmon temporarily suppressed APC production by the anterior kidney leukocytes. This response was similar to that of fish subjected to an acute handling stress. The other immune components we monitored, skin mucous lysozyme and respiratory burst activity, were not altered by electroshock. Investigations to de-
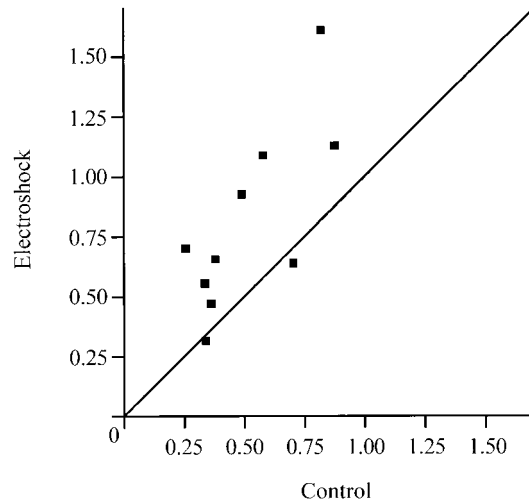
termine possible mechanisms responsible for the observed immune suppression suggest that electroshock indirectly inhibited APC generation by triggering a stress response. Our inquiries into the effects of electroshock on disease progression indicated no effects on infection severity or mortality but a small, yet statistically significant, acceleration in time to death.

We found that electroshock temporarily suppresses APC generation by anterior kidney leukocytes in juvenile spring chinook salmon. Although the difference between electroshocked and control fish in $IE_2$ was not statistically significant ($P = 0.06$), we believe this difference was biologically significant given the amount of variability in APC production reported for juvenile salmonids (Maule et al. 1987; 1989; Tripp et al. 1987). Similarities in the responses of electroshocked and handled fish from our trials and those reported in the literature (Maule et al. 1989) suggest that electroshock suppresses APC production indirectly. We also found that the ability of leukocyte suspensions to generate APC was not affected immediately after an in vitro electroshock, supporting our contention that electroshock affected APC generation indirectly.
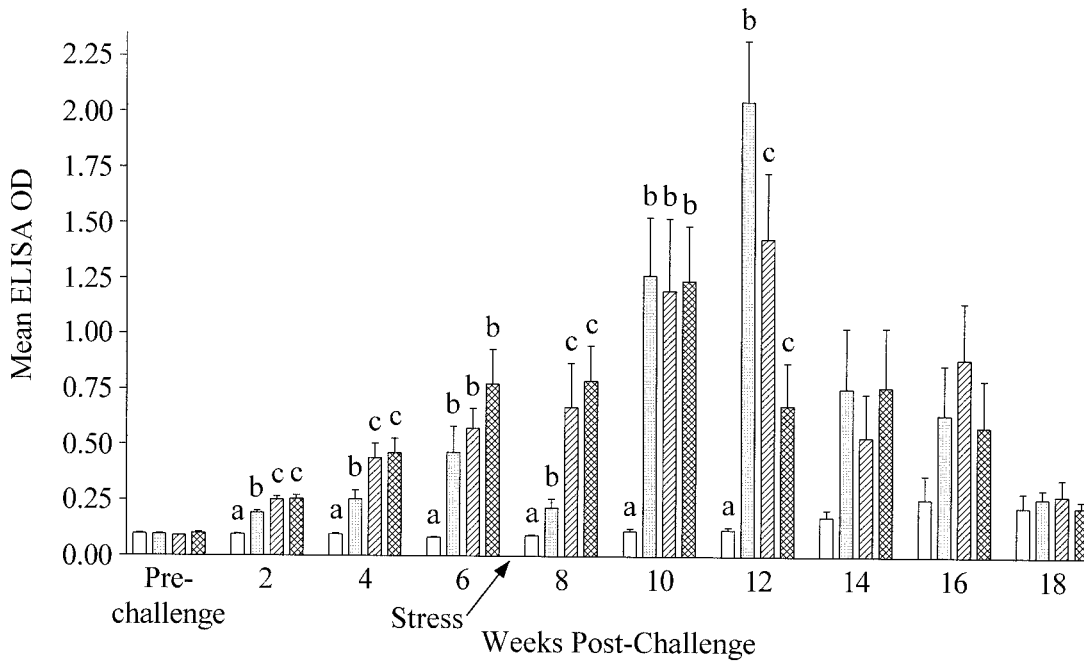
FIGURE 2.—Mean (+SE) ELISA OD (enzyme-linked immunosorbent assays of optical densities) of juvenile spring chinook salmon immediately before (prechallenge) and at 2-week intervals after the challenge in disease challenge 2; negative controls (clear bars) were challenged with sterile saline, whereas positive controls (gray bars) and other treatments were challenged with infection of *Renibacterium salmoninarum.* Approxomately 7 weeks after the challenge, fish were exposed to either a 2-s, 300-V, 50-Hz pulsed DC electroshock (stippled bars); a 30-s handling stress (crosshatched bars); or no stress (controls). Means represent data from two replicates that did not differ statistically and were pooled ($N = 10$), except for the negative controls that were represented by data from a single tank ($N = 5$). Means within a week sharing the same letter were not significantly different.

Suppression of APC production did not occur in all trials. The lack of expected changes at 3 h in electroshocked or handled fish in the third trial suggest that either the fish or conditions differed from those in other trials. Differences in size, developmental stage, or other physiological factors may have affected how these fish responded to stress. Water conductivity was somewhat lower at CRRL ($IE_1$ and $IE_3$) than SF ($IE_2$), but similarities in the results from $IE_1$ and $IE_2$ exclude this as an explanation for the results from $IE_3$. Other differences in water quality or unknown environmental factors may have also influenced the results. A caveat to our finding of APC suppression after electroshock is that we only used anterior kidney leukocytes. Given the dynamic nature of leukocyte populations after stress (Maule and Schreck 1990), our results may have differed if the responses of

TABLE 2.—For disease challenge 2, mean (SE) plasma cortisol and glucose concentrations in juvenile spring chinook salmon infected with *Renibacterium salmoninarum* as measured three times (3 h, 24 h, 72 h) after treatment with either a 2-s, 300-V, 50-Hz, 8-ms-pulse-width pulsed-DC electroshock, a 30-s handling stress (handling); or no stress (positive control). Means within a sample time without a letter in common differ significantly.

| Factor measured[a] | Treatment | Sample time | | |
|---|---|---|---|---|
| | | 3 h | 24 h | 72 h |
| Cortisol | Positive control | 6.7 (3.1) z | 13.8 (8.6) z | 15.0 (6.3) |
| | Electroshock | 50.9 (12.4) y | 28.0 (9.1) y | 16.3 (4.2) |
| | Handling | 24.7 (5.3) y | 11.5 (2.7) y | 6.4 (2.8) |
| Glucose | Positive control | 63.9 (3.7) z | 64.3 (4.5) | 53.9 (3.5) |
| | Electroshock | 106.3 (7.9) y | 74.5 (4.4) | 62.7 (2.2) |
| | Handling | 90.9 (6.7) y | 81.6 (11.7) | 58.1 (3.3) |

[a] Cortisol levels are reported as ng/ml plasma and glucose concentrations as mg/dL plasma.
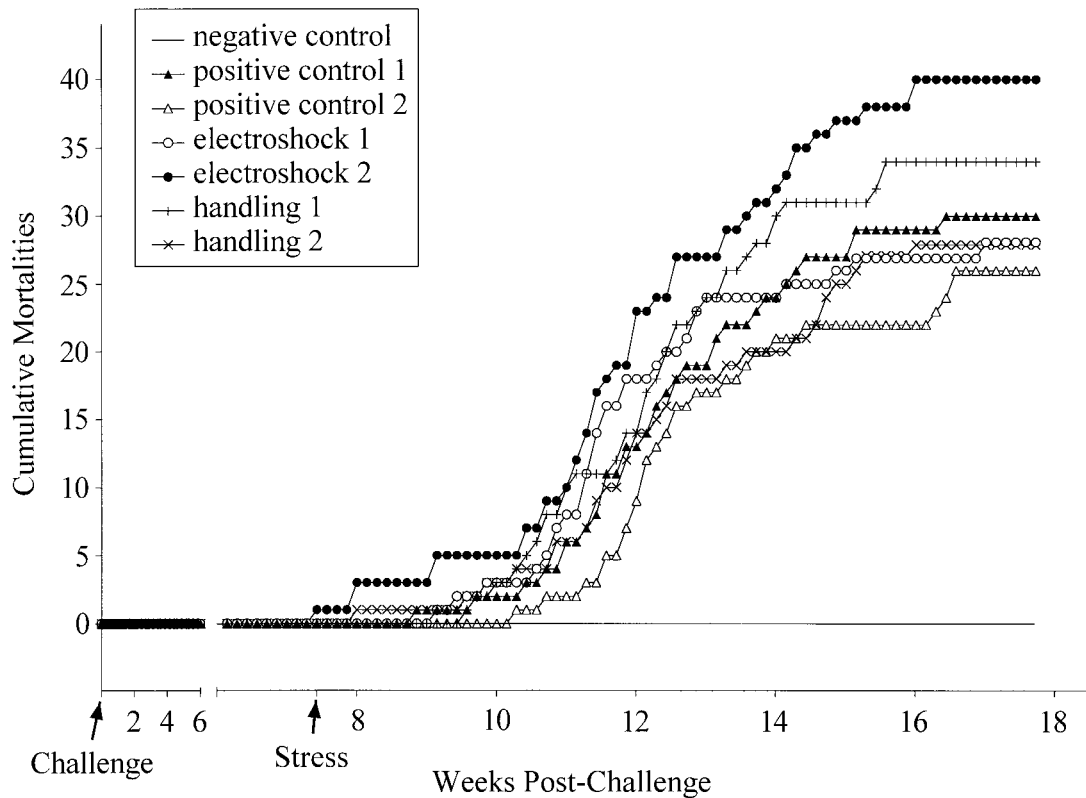
FIGURE 3.—Disease challenge 2 results presented as cumulative mortalities of juvenile spring chinook salmon after experimental infection with *Renibacterium salmoninarum*. Negative controls were challenged with sterile saline, whereas positive controls and other treatments were challenged with *R. salmoninarum*. Approximately 7 weeks after infection, fish were exposed to either a 2-s, 300-V, 50-Hz pulsed-DC electroshock; a 30-s handling stress; or no stress (controls). Each curve represents data from a separate tank.

splenic, thymic, or circulating leukocytes had been monitored.

The most obvious indirect mechanism of immune suppression would be via the stress response, which is known to alter the responses of several immune functions in salmonids (Angelidis et al. 1987; Maule et al. 1989; Möck and Peters 1990; Mazur and Iwama 1993; Thompson et al. 1993; Demers and Bayne 1997). We found that electroshocked and handled fish responded to stress in a similar fashion. Stress indicators were significantly elevated at 3 h, cortisol levels generally returning to control values within 24 h after stress and glucose concentrations generally recovering between 24 h and 7 d. The exception to this pattern was glucose levels from $IE_2$, which for unknown reasons did not respond to stress. We also found that cortisol levels at 7 d were somewhat variable—particularly the unexplained elevations in handled fish in one trial and controls in another—but we found no sign that electroshocked fish were

stressed after 7 d. The absence of direct effects on APC generation in conjunction with the responses of the stress indicators led us to conclude that suppression of APC generation 3 h after electroshock was due to elevated plasma cortisol concentrations in response to stress.

Skin mucous lysozyme levels in juvenile spring chinook salmon were not affected by exposure to electroshock. Recent work suggests plasma lysozyme levels in rainbow trout are altered after stress (Möck and Peters 1990; Demers and Bayne 1997). Our results may have differed because the electroshock was not of a sufficient duration or severity to elicit a response, or perhaps mucous lysozyme levels were altered but recovered within 3 h of electroshock. Responses may vary by species because basal lysozyme levels differ by species (Grinde et al. 1988; Lie et al. 1989). Blood and mucous lysozyme may also originate from different sources (e.g., neutrophils, macrophages, or eosinophilic granulocytes; Murray and Fletcher

1976; Lie et al. 1989; Sveinbjørnsson et al. 1996), which could respond differently to stress.

Exposure to electroshock had inconsistent effects on phagocyte respiratory burst activity. We also found, however, a lack of consistency among controls making it difficult to interpret the changes seen in electroshocked fish. Respiratory burst activity was highly variable in both trials, indicating either large amounts of natural variation or that activity levels were influenced by some unknown factor. We found equivocal evidence that electroshock directly effects respiratory burst activity; activity was enhanced in vitro immediately after electroshock in one of two trials. This contradicts reports of suppressed respiratory burst activity after stress (Angelidis et al. 1987; Thompson et al. 1993). If suppression occurred in vivo, the effect was short lived given the lack of differences at 3 h after electroshock in the immune function experiments. Based on the preponderance of evidence, however, we conclude that electroshock had no effect on respiratory burst.

We found little evidence in $CH_1$ that electroshock affects disease progression. The mean OD of electroshocked fish was greater than for control or handled fish 2 weeks after treatment, which seems to suggest that an electroshock exacerbated the RS infection. The mean OD of electroshocked fish at 2 weeks, however, did not differ from baseline controls (week 0), and the mean OD of electroshocked fish at 4 weeks did not differ from the controls at weeks 4 or 0. The lack of differences from baseline controls at any time demonstrates that electroshock did not alter progression of the infection.

Results from $CH_2$ suggest that time to death may be accelerated by electroshock. We also found that the mean OD of the electroshocked fish was significantly greater than that of infected, unstressed positive controls at 4 d after treatment (8 weeks postchallenge). The mean OD at 4 d after electroshock, however, was only slightly greater than pre-exposure levels (6 weeks postchallenge), whereas the mean OD of positive control fish dropped over the same period. These changes suggest that the difference at 4 d after treatment was due to a random change in the positive control levels rather than some effect of the electroshock. This conclusion is supported by the lack of differences between RS-infected treatments 10 weeks postchallenge. Further proof of the lack of influence of electroshock on RS infections was evident at week 12, when the mean OD of the positive controls was significantly greater than for electroshocked

fish. Mean ODs did not differ by treatment from week 14 through the end of the challenge, indicating that electroshock had no long-term effect on progression of the RS infection.

There was no clear effect of electroshock on cumulative mortality in RS-infected fish. The greatest number of mortalities was in one electroshock replicate, followed by a replicate of handled fish. Conversely, the replicates for each of these treatments had equal numbers of mortalities, which were intermediate to the two positive control replicates. We did find that the mean time to death of electroshocked fish was 5 d earlier than positive controls, and the handled fish were intermediate. The biological significance of this difference is unclear given that cumulative mortality of one replicate of electroshocked fish was intermediate between the two positive controls. It is also unclear what mechanism might be responsible for the acceleration in time to death. Although electroshock did induce a stress response, we found no effects on the severity of RS infection or numbers of mortalities. Disease resistance in salmonids is altered by stress (Maule et al. 1989; Pickering and Pottinger 1989), but less is known about the effects of stress on sick fish. Mesa et al. (2000) subjected RS-infected juvenile chinook salmon to a series of acute stress events and found no effect on infection levels, mortality, or mean time to death. Our results concur, other than for mean time to death, suggesting that acute stress has little effect on the severity of RS infections or any associated mortality.

Our research indicates that exposure to an electroshock briefly suppresses APC generation by head kidney leukocytes and has some minimal influence on disease progression in juvenile spring chinook salmon. The limited duration of immune suppression and lack of effect on mortality levels in RS-infected fish led us to conclude that electrofishing under the conditions of this study is a safe procedure in regards to immunity and disease. However, we consider our results to be preliminary and urge caution with their application to other scenarios. Because mortality and injury rates vary under different exposure conditions, it is possible that changes in voltage, pulse frequency, temperature, or other factors would yield different results.

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