AN ABSTRACT OF THE THESIS OF

Scott P. VanderKooi for the degree of Master of Science in Fisheries Science presented on June 16, 1999. Title: The Effects of Electroshock on Immune Function and Disease Progression in Juvenile Spring Chinook Salmon (*Oncorhynchus tshawytscha*).

Abstract approved: _____

Alec G. Maule and Carl B. Schreck

Electrofishing is a sampling tool commonly used by fisheries researchers. While much is known about the effects of electroshock on fish physiology, consequences to the immune system and disease progression have not received attention. To understand the effects of electroshock on immune function we undertook a comparison of electroshock and handling stress in regards to selected immune functions and disease progression in juvenile spring chinook salmon (*Oncorhynchus tshawytscha*). The handling stress treatment was included to insure the responsiveness of the fish. Our objectives were to determine the effects of electroshock on immune function, determine the mechanism of any observed alteration, and to determine the effects of electroshock on disease progression. Skin mucous lysozyme concentrations were not affected by exposure to electroshock. Respiratory burst activity may be enhanced in leukocytes immediately after an *in vitro* electroshock. Any effect *in vivo,* however, appears to be brief given the lack of differences observed 3 h after exposure. The specific immune response, measured as the ability of anterior kidney leukocytes to

generate antibody producing cells (APC), was suppressed 3 h after electroshock, 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 elevation of plasma cortisol concentrations. The ability to generate APC may be suppressed 7 d after electroshock, but it is not evident what mechanism is responsible for this suppression. There was some evidence that the progression of a *Renibacterium salmoninarum* (RS) infection was altered after exposure to an electroshock. Exposure to electroshock did not have a clear affect on the severity of infection or the number of mortalities, but may have accelerated the time to death in infected fish that died. The limited duration of specific immune suppression and lack of effect on mortality in RS infected fish lead us to conclude that electrofishing under the conditions we tested is a safe procedure in regards to immunity and disease.

The Effects of Electroshock on Immune Function and Disease Progression in Juvenile Spring Chinook Salmon (*Oncorhynchus tshawytscha*).

by

Scott P. VanderKooi

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Contribution of Authors

Dr. Alec G. Maule and Dr. Carl B. Schreck were involved in the design and analysis of all experiments conducted for this study as well as the writing of the manuscript.

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The Effects of Electroshock on Immune Function and Disease Progression in Juvenile Spring Chinook Salmon (*Oncorhynchus tshawytscha*).

Introduction

The immediate physiological effects of electroshock on fish are well documented. Overt physical damage resulting from exposure to alternating current (AC) or direct current (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 plasma catecholamine (Mitton and McDonald 1994), lactic acid (Schreck et al. 1976; Mesa and Schreck 1989), and glucose levels (Schreck et al. 1976; Barton and Grosh 1996). Another physiological effect of electroshock is the elevation of plasma cortisol levels, indicative of a stress response being triggered (Schreck et al. 1976; Mesa and Schreck 1989; Maule and Mesa 1994; Mitton and McDonald 1994; Barton and Grosh 1996).

The extent of physical damage and mortality can vary according to many factors including the type of current used. The prevailing opinion is that the levels of mortality and injury are highest with AC, intermediate with pulsed DC, and lowest with smooth DC (Lamarque 1990). Recent studies support this view showing higher spinal injury rates in rainbow trout (*Oncorhynchus mykiss*) exposed to pulsed DC than those exposed to smooth DC (McMichael 1993; Dalbey et al. 1996). On the contrary,

Hollender and Carline (1994) found no significant difference in injury rates among brook trout (*Salvelinus fontinalis*) exposed to AC and pulsed DC. Mortality of chinook salmon (*O. tshawytscha*) fingerlings exposed to pulsed DC rises with increases in exposure duration, pulse frequency, and water temperature (Collins et al. 1954). Increased injury rates in rainbow trout have been associated with higher pulse frequencies (McMichael 1993; Sharber et al. 1994). The frequency and severity of injuries due to electroshock may also vary by species (McMichael 1993; Sharber et al. 1994; Bardygula-Nonn et al. 1995; Sharber and Carothers 1988).

Some sub-lethal effects of electroshock that may have negative implications have been investigated. Dwyer and White (1995) reported that weight gain was reduced in rainbow trout 35 d after a pulsed-DC electroshock. Similar results were observed in arctic grayling (*Thymallus arcticus*) and Yellowstone cutthroat trout (*O. clarki bouvieri*) 28 d after electroshock. There is also evidence of reduced growth over longer periods of time. Gatz et al. (1986) found that growth rates were reduced in age-1 and age-2 rainbow trout and brown trout (*Salmo trutta*) that had been electroshocked two to seven times in a 12-month period. Electroshock has also been shown to alter behavior and feeding rates in cutthroat trout (*O. clarki*) (Mesa and Schreck 1989).

Other physiological changes that could affect the health and survival of fish after exposure to electroshock have not been examined. An aspect of physiology critical to the health and survival of an organism is the proper function of its immune

system. The immune system helps maintain homeostasis by providing a means for an individual to protect itself from harm by exogenous (e.g., viruses, bacteria, fungi, parasites, and inorganic substances) and endogenous threats (e.g., cancerous cells and tumors).

The epithelial cells covering the skin, gills, and gut of a fish are the first line of defense against foreign invaders. The defensive nature of this barrier is enhanced by the mucous layer secreted by these cells. The mucus contains many specific and non-specific immune factors including antibody and lysozyme which can destroy or disable pathogens on the exterior of a fish (Alexander and Ingram 1992). Once an invader is inside a fish the immune system relies first on the quick reactions of non-specific factors to contain and eliminate the threat. C-reactive protein, complement, lysozyme, and other non-cellular factors act or interact to agglutinate, opsonize, or destroy pathogens (Alexander and Ingram 1992). Non-specific immune cells attack invaders with chemicals like oxygen free radicals (respiratory burst) or more directly by engulfing and destroying them internally (phagocytosis) (Secombes and Fletcher 1992). Phagocytosis can trigger some functions in the next level of defense--the specific immune response. Phagocytes present foreign antigen to T-cells which then become activated and differentiate into helper T-cells (T_H-cells). The T_H-cells activate B-cells which differentiate into plasma cells which in turn produce antibody specific to that foreign antigen. Specific antibody can neutralize toxins, agglutinate or opsonize

foreign cells, and act with complement to lyse those cells or facilitate their uptake by phagocytes (Janeway and Travers 1994).

One of the primary responses of fish to stress is the elevation of circulating corticosteroid concentrations (Mazeaud et al. 1977). Elevated plasma cortisol levels are known to suppress the production of antibody-producing cells by splenic and pronephric lymphocytes of juvenile coho salmon (*O. kisutch*) both *in vitro* (Tripp et al. 1987) and *in vivo* (Maule et al. 1987). Reduced disease resistance is also associated with elevated plasma cortisol levels (Maule et al. 1987; Pickering and Pottinger 1989). Similar findings have been seen in fish after acute or chronic stress. The ability to produce specific antibody-producing cells and resist disease were reduced in juvenile chinook salmon 4 h after an acute handling stress (Maule et al. 1989). Chronic crowding stress of juvenile Atlantic salmon (*S. salar*) also resulted in lowered production of specific antibody-producing cells (Mazur and Iwama 1993). Other immune responses are altered by stress. Plasma lysozyme activity in rainbow trout increased after a handling stress of 30 min or less (Möck and Peters 1990; Demers and Bayne 1997). Möck and Peters (1990), however, also found reduced plasma lysozyme activity after a longer stress (2 h of transportation). Phagocyte respiratory burst activity was also reduced after stress in Atlantic salmon (Thompson et al. 1993) and rainbow trout (Angelidis et al. 1987).

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 which likely results, at least temporarily, in altered responses of certain immune functions. Therefore, it can be hypothesized that an electroshock would alter the immune response in fish, at least indirectly, through elevation of plasma cortisol levels. In the following research, we undertook a comparison of electroshock and handling stress in regards to selected immune functions and disease progression in juvenile spring chinook salmon. For our experiments we selected a pulsed DC electroshock (300 V, 50 Hz) with an exposure duration of 2 s. These settings were selected because they are similar to those used by researchers investigating the impacts of electrofishing as well as in field research in the Pacific Northwest. Exposure duration was necessarily brief due to the close proximity of the electrodes. Preliminary experimentation indicated a 2 s exposure was sufficient to achieve electronarcosis without mortality or visible injury.

The first objective of this study was to determine the effects of electroshock on the responses of selected immune functions and to place any alteration in the context of a stressor known to change these responses. Our second objective was to determine the mechanism of any alteration (i.e., to separate possible effects at the organism level from those directly on leukocytes) by monitoring the stress response of electroshocked fish and the responses of immune cells exposed to an electroshock *in vitro*. Our final objective was to determine the effects of electroshock on disease progression and again place any change in the context of a stressor known to alter disease resistance.

Methods

Experimental animals

Juvenile spring chinook salmon were obtained from either Oregon State University's Fish Performance and Genetics Laboratory, Smith Farm, Corvallis, Oregon (SF) or Little White Salmon National Fish Hatchery (NFH) Washington, U.S.A. (LWS). Disease challenges were conducted at Oregon State University's Salmon Disease Laboratory, Corvallis, Oregon (SDL) 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 61-cm circular fiberglass tanks with a water depth of 45 cm and at SDL in 100-L cuboidal fiberglass tanks with a water depth of 27 cm all provided with flow-through well water at 12 °C, ambient conductivity 530 - 533 μS/cm. Fish for experiments conducted at CRRL were maintained in either 61-cm or 86-cm circular fiberglass tanks with water depths of 45 cm and 71 cm, respectively, 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 daily to satiation.

Immune function experiments

Immune competence was assessed in three experiments (immune experiments I, II, and III) which monitored selected immune functions several times after

treatments of electroshock, handling stress, or no stress (control). The handling stress treatment was included to insure the responsiveness of the fish in terms of stress and immune responses. Immune experiments I, II, and III were conducted in August 1996, September 1996, and August 1997 respectively. Fish were stocked randomly in 61-cm circular, fiberglass tanks at 20 fish per tank for immune experiment I (mean fork length 107 mm) and 25 fish per tank for immune experiments II (mean fork length 99 mm) and III (mean fork length 167 mm). Fish were allowed to acclimate for two weeks prior to the start of each experiment. Tanks were assigned treatments by random drawing. Following acclimation, fish in duplicate tanks were treated with a pulsed DC electroshock (300 V, 50 Hz) for 2 s or a handling stress of capture by dip net and suspension out of water for 30 s or no treatment (control). Fish were sampled at 3 h, 24 h, and 7 d after treatment. At each sample time four (immune experiment I) or five (immune experiments II and III) fish were rapidly netted from each tank and transferred into a lethal dose of buffered MS-222 (200 mg/L). After fish were fully anesthetized the fork length and weight of each was recorded.

In immune experiment I skin mucus was collected to monitor skin mucous lysozyme levels. One sample of skin mucus was collected from each fish by wiping a 10-µl inoculation loop 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. Each sample was mixed with the buffer by twirling the loop back and forth between fingers approximately 30

times. Samples of skin mucus were stored at -80 °C. Lysozyme concentrations were determined by analysis of skin mucous samples using a micro plate method developed from the method of Litwack (1955) as modified by Sankaran and Gurnanai (1972), following Muona and Soivio (1992). Briefly, 25 µl of the sample mucus-buffer mixture was combined with 250 µl of 0.25 g/L *Micrococcus lysodeikticus* in 0.02 M acetate buffer suspension in the wells of a 96-well micro plate. The sample and acetate buffer solution were incubated at 37 °C and the absorbances were measured with a spectrophotometer at 450 nm at time 0 and again after 20 min. The standards run on each plate consisted of a dilution series of 0, 6, 9, and 15 µg hen egg-white lysozyme per ml of 0.04 M phosphate buffer. There were four replicates of 25 µl standard in 250 µl of *Micrococcus*-acetate suspension per concentration run on each plate. A standard curve was constructed from the mean change in absorbance for each concentration, after accounting for the blank. The concentration of lysozyme in a sample, reported in µg/ml hen egg-white lysozyme, is proportional to the decrease in absorbance over time.

The stress response of fish was monitored by measuring plasma cortisol and glucose concentrations. The caudal peduncle was severed and blood was collected with heparin-coated capillary tubes. Plasma was separated from whole blood by centrifugation; samples were stored at -20 °C. Cortisol concentrations were determined from analysis of plasma samples by the radio-immunoassay method described by Foster and Dunn (1974) modified by Redding et al. (1984). Glucose

concentrations were determined from analysis of plasma samples with a glucose/hexokinase colorimetric assay (Sigma Diagnostics, St. Louis, Missouri, USA).

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 gently pushing tissue and media through 100-μm mesh with the plunger of a 1-ml tuberculin syringe onto a sterile petri dish. The mesh was then washed with fresh TCM and the suspension was aspirated with a Pasteur pipette and placed in a 5-ml sterile capped tube on ice. The suspension was centrifuged for 10 min at 500 × g (4 °C). The supernatant was aspirated and discarded, cells were then resuspended in 0.5 or 1.0 ml fresh TCM. After allowing tissue debris to settle, a 10-μl aliquot of suspension was removed and combined with 150 μl of TCM and 40 μl of 0.4% (w/v) trypan blue in a well of a 96-well micro plate. The suspension was gently mixed with a Pasteur pipette and loaded onto a haemocytometer where viable leukocytes were counted under a compound microscope. Based on the cell count, the tissue suspension was diluted to a working concentration of 2×10^7 cells/ml. A 50-μl aliquot of the working cell suspension was placed in a well of a 96-well micro plate, for a concentration of 10^6 cells per well, with 50 μl TCM containing the antigen trinitrophenol-lipopolysaccharide (TNP-LPS). Production of antibody producing cells was assessed *in vitro* using the passive

haemolytic plaque assay described by Tripp et al. (1987) and modified by Slater et al. (1995). The only alteration from the method of Slater et al. (1995) was that diluted (1:10) rainbow trout serum was used as the complement source rather than steelhead serum.

Phagocytes were isolated to measure their respiratory burst activity using a modification of the method 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 × g (4 °C), the supernatant was aspirated and discarded, and cells were resuspended in ice cold tissue culture medium 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 micro plate, covered, and incubated for 1 h at 17 °C to allow phagocytes to adhere. Cells were washed twice with 0.1% TCM to remove non-adherent cells. Respiratory burst activity was assessed with a modification of Secombes' (1990) nitroblue tetrazolium (NBT) assay for detection of intracellular superoxide anion (O_2^-). A 100-µl aliquot of NBT solution consisting of 1 mg/ml NBT, 1 µl/ml of a 1 µg/ml solution of phorbol myristate acetate in ethanol, and 299 µg/ml superoxide dismutase in phenol red-free Hank's Balanced Salt Solution was added to each well of adherent cells. The plates were covered and incubated for 2 h at 17 °C. After incubation the supernatant was aspirated and discarded. Cells were fixed with the addition of 100 µl methanol to each

well then washed three times with 100-µl aliquots of 70% methanol and air dried. Each well received 120 µl 2M KOH and 140 µl DMSO then the plate was shaken for 60 s and absorbances were measured with a spectrophotometer at 630 nm.

In immune experiment II we measured plasma glucose and cortisol concentrations and the production of APC. The protocols from immune experiment I were followed to collect blood, isolate, store , and analyze plasma as well as harvest and isolate anterior kidney leukocytes. Production of APC was assessed following the method of Slater et al. (1995) without modification. Immune experiment III followed the methods described for immune experiment I for sample collection and tissue preparation with one exception. Production of APC was assessed following the method of Slater et al. (1995) without modification.

In vitro experiments

We investigated the direct effects of electric current on leukocyte responses in two experiments (*in vitro* experiments I and II) in September 1997. Cells were exposed to an electroshock *in vitro* to determine the effects of this treatment on APC production and respiratory burst activity independent of the stress response. Ten fish (mean fork length 171 mm) for each *in vitro* experiment were rapidly netted and transferred into a lethal dose of buffered MS-222 (200 mg/L). Blood was collected and plasma was isolated, stored and analyzed for cortisol and glucose concentrations as described for immune experiment I. To assess APC production anterior kidney

leukocytes were harvested and suspended in TCM using the methods described for immune experiment I. The suspensions were centrifuged for 10 min at 500 × g (4 °C), the supernatant was aspirated and discarded, and cells were resuspended in 2 ml TCM. Duplicate 1-ml aliquots of each suspension were placed in small lengths of dialysis tubing (molecular weight cutoff: 6-8000) that had been soaked and rinsed with distilled-deionized water then rinsed with TCM and sealed at one end with a 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 as treatment and the other as control, thus each fish acted as its own control.

Five loaded sections of tubing at a time were suspended from a piece of PVC pipe with equally spaced lengths of string. Each sample was suspended just under the surface of the water in a 61-cm circular fiberglass tank filled with 9 °C well water, ambient conductivity 70 μS/cm. A small glass weight was attached to the lower clip to keep each sample submerged vertically in the water column. Electrodes were placed on opposite sides of the tank and the length of pipe positioned across the tank so that current flow would be perpendicular to the pipe and the samples midway between the electrodes. Treatment samples were exposed to a pulsed DC electroshock (300 V, 50 Hz) for 2 s, removed from the tank, and immediately placed on ice. Control samples were treated identically with the exception of the electroshock. The tubing of each sample was cut just above the fluid, the suspension was aspirated and placed in a 5-ml sterile capped tube on ice. Some fresh TCM was added to the tubing

to remove cells, aspirated, and added to the suspension. Each suspension was centrifuged for 10 min at 500 × g (4 °C), the supernatant was aspirated and discarded, and cells were resuspended in 0.5 ml TCM. Leukocytes were quantified and diluted as described for immune experiment I. Production of antibody producing cells was assessed following the method Slater et al. (1995). Phagocytes were isolated and respiratory burst activity measured as described for immune experiment I.

Disease challenges

The effect of electroshock on the progression of disease in juvenile chinook was tested in two experiments, first in January 1997 (disease challenge I) and again in August 1997 (disease challenge II), with fish infected by *Renibacterium salmoninarum* (RS), the pathogenic cause of bacterial kidney disease (BKD). In disease challenge I fish (mean fork length 135 mm) with a natural RS infection were stocked at 30 per 100-L cuboidal fiberglass tank and were allowed to acclimate for at least 2 weeks. The pathogen was identified by the combination of low level mortality and gross pathology with later confirmation by laboratory analysis as described below. Since all fish were stocked from the same source it can be assumed that all had been exposed to RS and the incidence of infection was the same in all tanks at the beginning of the experiment. Each of seven treatments was assigned to triplicate tanks by random drawing. Following acclimation, fish were treated with an electroshock or a handling stress or no treatment (control) as described for immune experiment I. The handling

stress treatment was included to insure the responsiveness of the fish in terms of stress response and disease progression. Control tanks were divided among three sample times: the beginning of the experiment (day 0), 2 weeks, and 4 weeks. Electroshock and handling-stress tanks were sampled at either 2 or 4 weeks post stress. Mortalities were removed daily and tallied. At each sample time all fish from a tank were rapidly netted and transferred into a lethal dose of buffered MS-222 (200 mg/L). Fork lengths and weights of all fish were recorded, then kidney and spleen tissues were collected from each using methods to avoid cross contamination of samples (Pascho et al. 1987). Samples were stored at -80 °C. The confirmation of mortality due to RS as well as RS infection levels were determined by analysis of kidney samples by an enzyme linked immunosorbent assay (ELISA). Preparation and analysis of samples followed the protocol for the ELISA II described by Pascho et al. (1991). Analysis by ELISA yields optical density (OD) values which translate to severity of infection as follows; values up to 0.2 are low-level infections, 0.2 to 1.0 moderate, and ODs greater than 1.0 are high-level infections.

Fish for disease challenge II (mean fork length 109 mm) were stocked at 130 fish per 86-cm circular fiberglass tank and allowed to acclimate for 2 weeks. After acclimation and just prior to challenge 7 fish from each tank were rapidly netted, placed in anesthetic, sampled, and analyzed as described for disease challenge I to determine baseline disease levels. Fish were exposed to RS by a modification of the waterborne immersion challenges described by Murray et al. (1992) and Elliott and

Pascho (1995). The bacterium used for this experiment was RS, stock isolate DWK-90. This isolate was originally cultured from the kidney of a chinook salmon smolt at Dworshak NFH, Idaho., U.S.A. by personnel at the USGS Western Fisheries Research Center, Seattle, Washington. Two 1-ml aliquots of the isolate were added to 1 L modified KDM-2 broth medium (Elliott and Pascho 1995) and incubated with stirring at 15 °C for 8 d. The bacteria were harvested by centrifugation at 3400 × g for 40 min at 15 °C and resuspended in sterile peptone saline (Murray et al. 1992) to a turbidity of 1.0 OD at 520 nm. The concentration of RS in the challenge suspension was determined to be 9.5×10^8 bacteria/ml by culture on KDM-2 agar plates for a concentration of 9.5×10^5 bacteria/ml during the immersion challenge. Each of four treatments were assigned to duplicate tanks by random drawing. The immersion challenge was conducted by turning off water flow to all tanks and lowering the water volume to 75 L. Oxygen was maintained in all tanks with air flow from airstones. The fish in the treatment and positive control tanks were challenged with 75 ml of bacteria-saline suspension for 24 h. Negative control fish were challenged with the addition of 75 ml of sterile peptone saline for the same period of time. Water flow was restored to all tanks at the end of the challenge. Every 2 weeks 7 fish from each tank were sampled as described above to monitor disease progression. Seven weeks after immersion challenge, fish were treated with an electroshock or a handling stress or no treatment (control) as described for immune experiment I. Physiological response to treatments was monitored at 3 h, 24 h, and 72 h post stress. Five fish from

each tank were rapidly netted and transferred into a lethal dose of buffered MS-222 (200 mg/L). Blood was collected and plasma isolated, stored and analyzed for cortisol and glucose concentrations as described in immune experiment I. After the stress treatments, disease progression was monitored as described above at eight weeks after immersion challenge and continued to be monitored at 2-week intervals for another 10 weeks.

Data analyses

Replicate tanks from immune experiments were tested for differences by two-way t-tests or Wilcoxon rank-sum test. Replicates from disease challenges were tested for differences by general linear models procedure (GLM) F-test (SAS Institute 1994) or Wilcoxon rank-sum test. Results from replicates not found to be different were pooled for further analysis. Box-plots and residual plots of the data were constructed to identify the presence of outliers and examine relative spreads to recognize data in need of transformation. Most data from immune experiments were analyzed by the GLM F-test. Multiple comparisons were made using the least significant difference (LSD). Antibody producing cell data from all immune experiments and skin lysozyme data from immune experiment III were analyzed using the non-parametric Kruskal-Wallis test with multiple comparisons by LSD where appropriate. Non-parametric techniques were used with these data due to large numbers of outliers in the APC data and numerous fish with no detectable lysozyme in immune experiment III. Data from

the *in vitro* experiments were analyzed by paired t-test. Data from disease challenges were analyzed by t-test, Kruskal-Wallis test, or GLM F-test with multiple comparisons using LSD.

Results

Immune function experiments

Data from replicates in all immune experiments did not differ (t-test or Wilcoxon rank-sum test, $P > 0.05$) so were pooled for further analysis. Skin mucous lysozyme concentrations did not vary by treatment or sample time in immune experiment I (GLM F-test, $P = 0.86$) (Figure 1 A). We found that skin mucous lysozyme levels differed by treatment or sample time in immune experiment III (Kruskal-Wallis test, $P = 0.0005$) (Figure 1 B). Lysozyme levels in handled fish 7 d after stress were significantly lower than the control and electroshocked fish ($P \leq 0.034$) (Figure 1 B).

Respiratory burst activity, measured as the amount of NBT reduced, differed by treatment or sample time in immune experiments I and III (GLM F-test on ln transformed data, $P \leq 0.0002$). All NBT values were multiplied by 100 prior to transformation to avoid negative values that result when ln transforming values > 0 and < 1 (Sokal and Rohlf, 1981). We found suggestive evidence in immune experiment I that respiratory burst activity was lower in electroshocked fish than in handled fish ($P = 0.048$) 7 d after stress, but neither treatment differed from controls (Figure 2 A). In immune experiment III there was strong evidence that respiratory burst activity was higher in electroshocked fish compared to control and handled fish 7

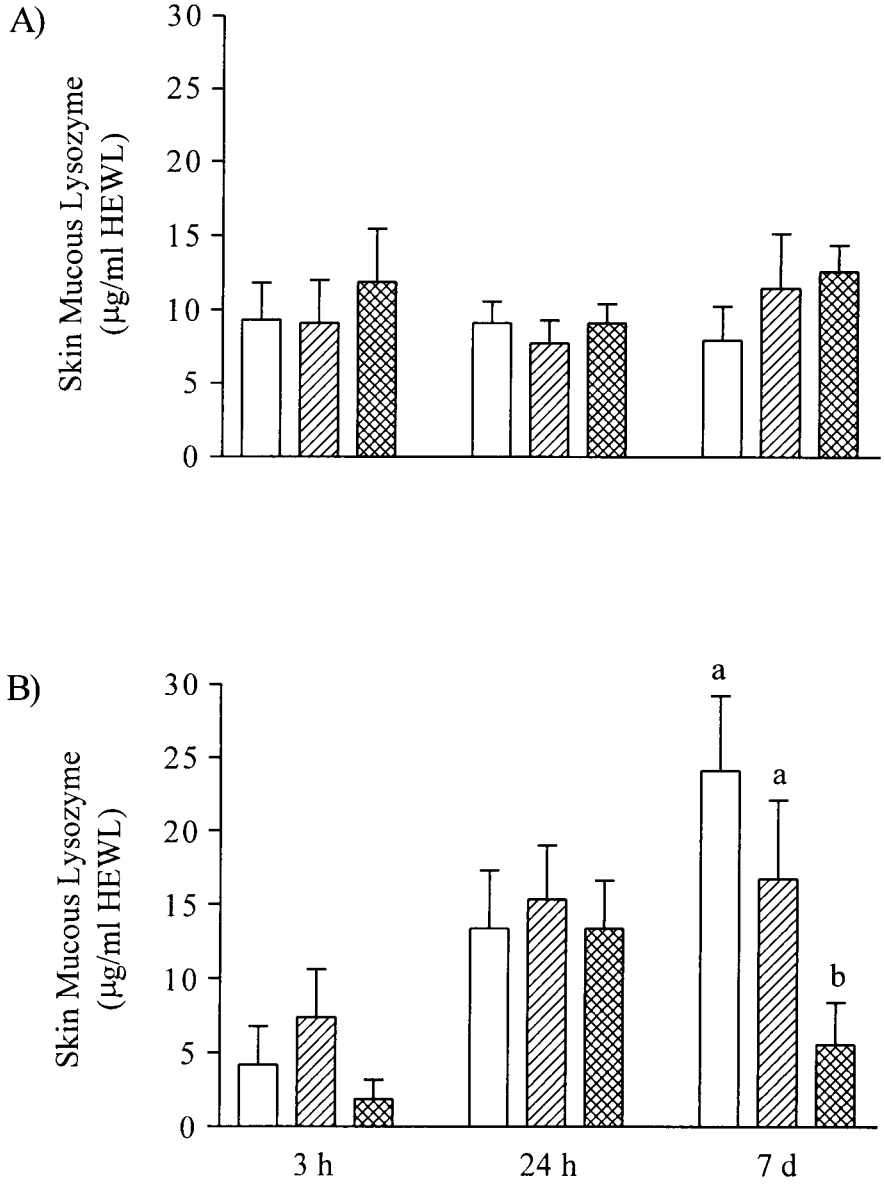

Figure 1. Mean (+ SE) skin mucous lysozyme in juvenile spring chinook salmon exposed to a 2-s, 300-V, 50-Hz pulsed DC electroshock (▨), 30-s handling stress (▩), or no stress (▭) from immune experiments I (A) and III (B). Means represent data from two replicates that did not differ statistically and were pooled. Results are given for three times after stress. Means within a time period without letters in common differ significantly.

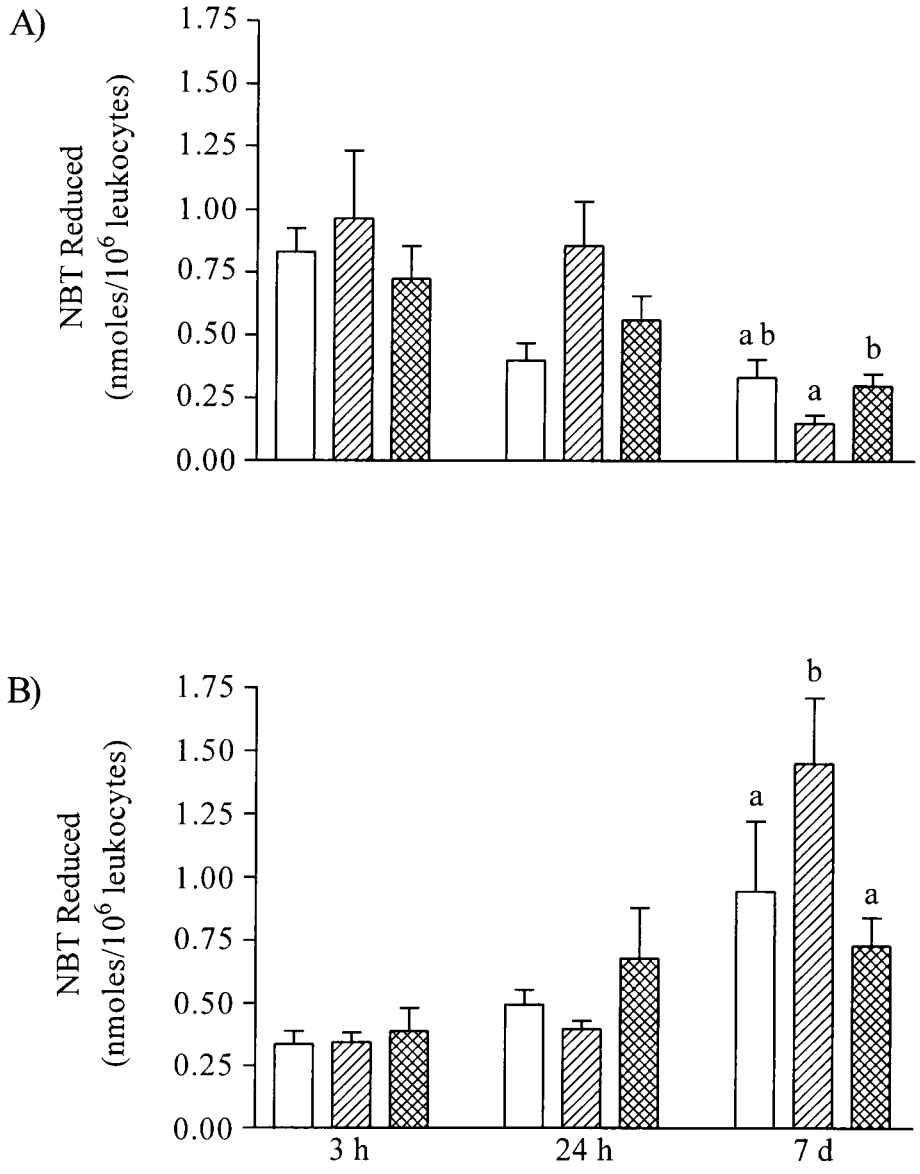

Figure 2. Mean (+ SE) NBT reduced as a measure of respiratory burst activity in juvenile spring chinook salmon exposed to a 2-s, 300-V, 50-Hz pulsed DC electroshock (▨), 30-s handling stress (▩), or no stress (□) from immune experiments I (A) and III (B). Means represent data from two replicates that did not differ statistically and were pooled. Results are given for three times after stress. Means within a time period without letters in common differ significantly.

d after stress ($P \leq 0.016$) (Figure 2 B). We also found that controls at 7 d were significantly higher than controls at 3 h ($P = 0.004$).

Analysis indicated that the ability to generate APC varied by treatment or sample time in immune experiments I, II, and III (Kruskal-Wallis test, $P = 0.021$, $P = 0.043$, and $P = 0.0001$ respectively). The number of APC produced was significantly lower in electroshocked fish compared to control or handling groups ($P \leq 0.004$) 3 h following stress in immune experiment I (Figure 3 A). No differences were detected 24 h after treatment. Control levels at 3 h and 24 h in experiment I were 74 and 18 APC / 10^6 leukocytes respectively. In immune experiment II the handled group produced significantly fewer APC than controls 3 h after stress ($P = 0.004$). There was also suggestive, but inconclusive evidence that electroshocked fish were different from controls at the same time ($P = 0.062$). Treatment and control groups did not differ 24 h or 7 d after stress ($P \geq 0.12$) (Figure 3 B). Control levels at 3 h, 24 h, and 7 d in experiment II were 683, 816, and 384 APC / 10^6 leukocytes respectively. In immune experiment III the number of APC produced by electroshocked fish was significantly lower than control and handled fish ($P = 0.035$) 7 d after stress (Figure 3 C). Control levels at 3 h, 24 h, and 7 d in experiment III were 75, 168, and 229 APC / 10^6 leukocytes respectively.

Both electroshock and 30-s of handling induced a stress response in juvenile spring chinook salmon as evidenced by elevated plasma cortisol levels 3 h after stress in all three immune experiments. There was convincing evidence that plasma cortisol

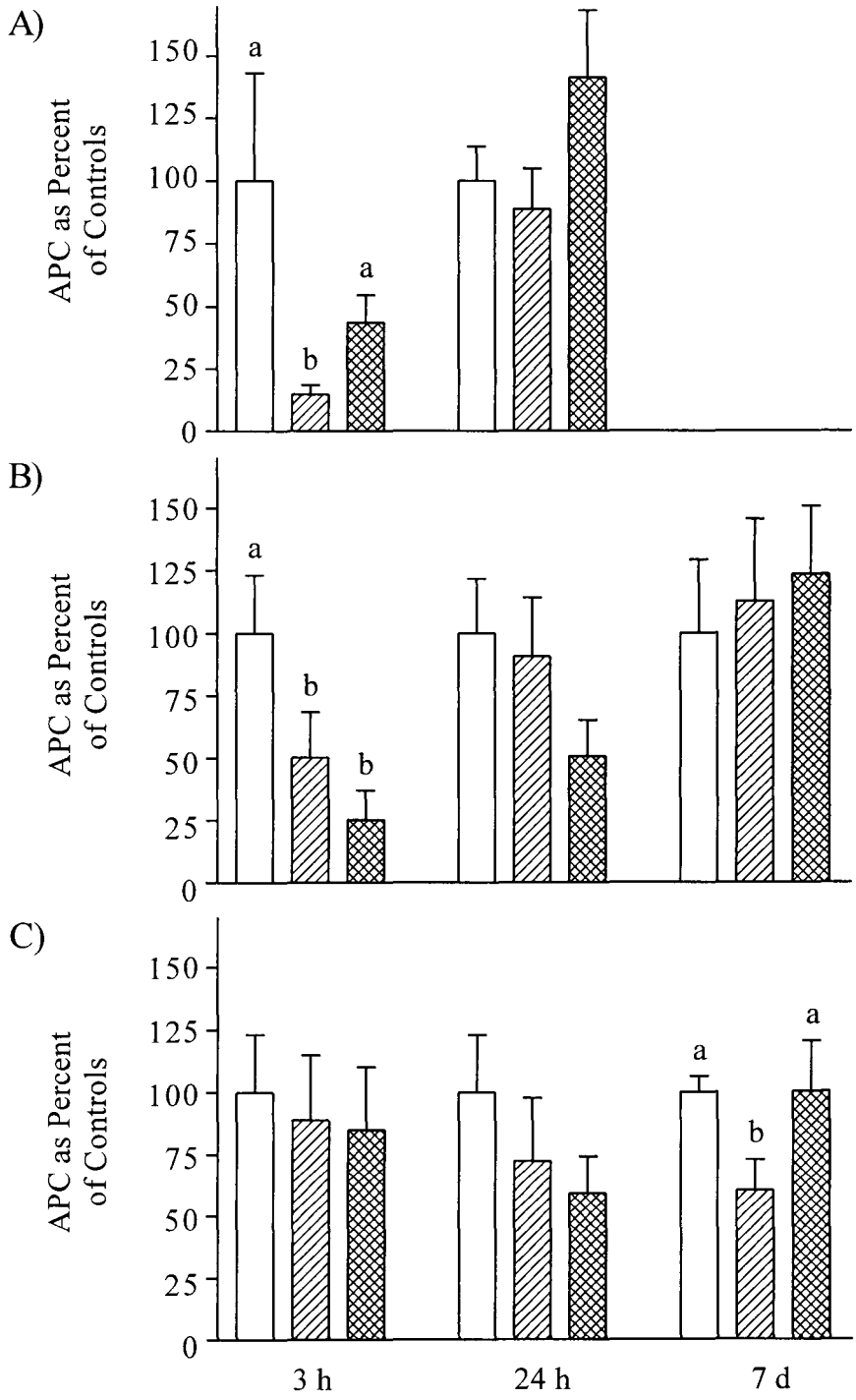

Figure 3. Mean (+ SE) antibody producing cells (APC) produced per 10^6 leukocytes from juvenile spring chinook salmon exposed to a 2-s, 300-V, 50-Hz pulsed DC electroshock (▨), 30-s handling stress (▩), or no stress (□) from immune experiments I (A), II (B), and III (C). Means represent data from two replicates that did not differ statistically and were pooled. Results are expressed as percentage of controls due to large amounts of inter-assay variation and are given for three times after stress. Means within a time period without letters in common differ significantly.

levels varied by treatment or sample time in immune experiments I, II (GLM F-test on natural log (ln) transformed data, $P \leq 0.002$) and III (GLM F-test on square-root transformed data, $P = 0.0001$). In immune experiment I cortisol levels 3 h after stress were significantly higher than controls in electroshocked and handled fish ($P \leq 0.009$). We found that both treatments returned to resting levels within 24 h of stress (Figure 4 A). After 7 d cortisol levels in electroshocked fish were significantly higher than controls ($P = 0.049$), but not different from handled fish (Figure 4 A). Cortisol concentrations were also significantly higher than controls in electroshocked and handled fish ($P \leq 0.0005$) 3 h after stress in immune experiment II. There was also suggestive, though inconclusive evidence that electroshocked fish were higher than handled fish at the same time ($P = 0.064$) (Figure 4 B). We found that cortisol concentrations in electroshocked fish at 24 h were still elevated above controls ($P = 0.034$), but were not different than handled fish. At 7 d after stress, cortisol levels in electroshocked fish did not differ from controls (Figure 4 B). Compared to controls, cortisol levels in immune experiment III were significantly higher 3 h after stress in fish subjected to electroshock or handling ($P \leq 0.004$). Neither treatment differed from controls 24 h after stress (Figure 4 C). Cortisol concentrations were significantly higher in controls than in electroshock or handled fish ($P \leq 0.008$) 7 d post stress. Controls at 7 d, however, were significantly higher than those at 24 h ($P = 0.008$) while the same comparison among electroshock and handling groups found no differences ($P \geq 0.53$) (Figure 4 C).

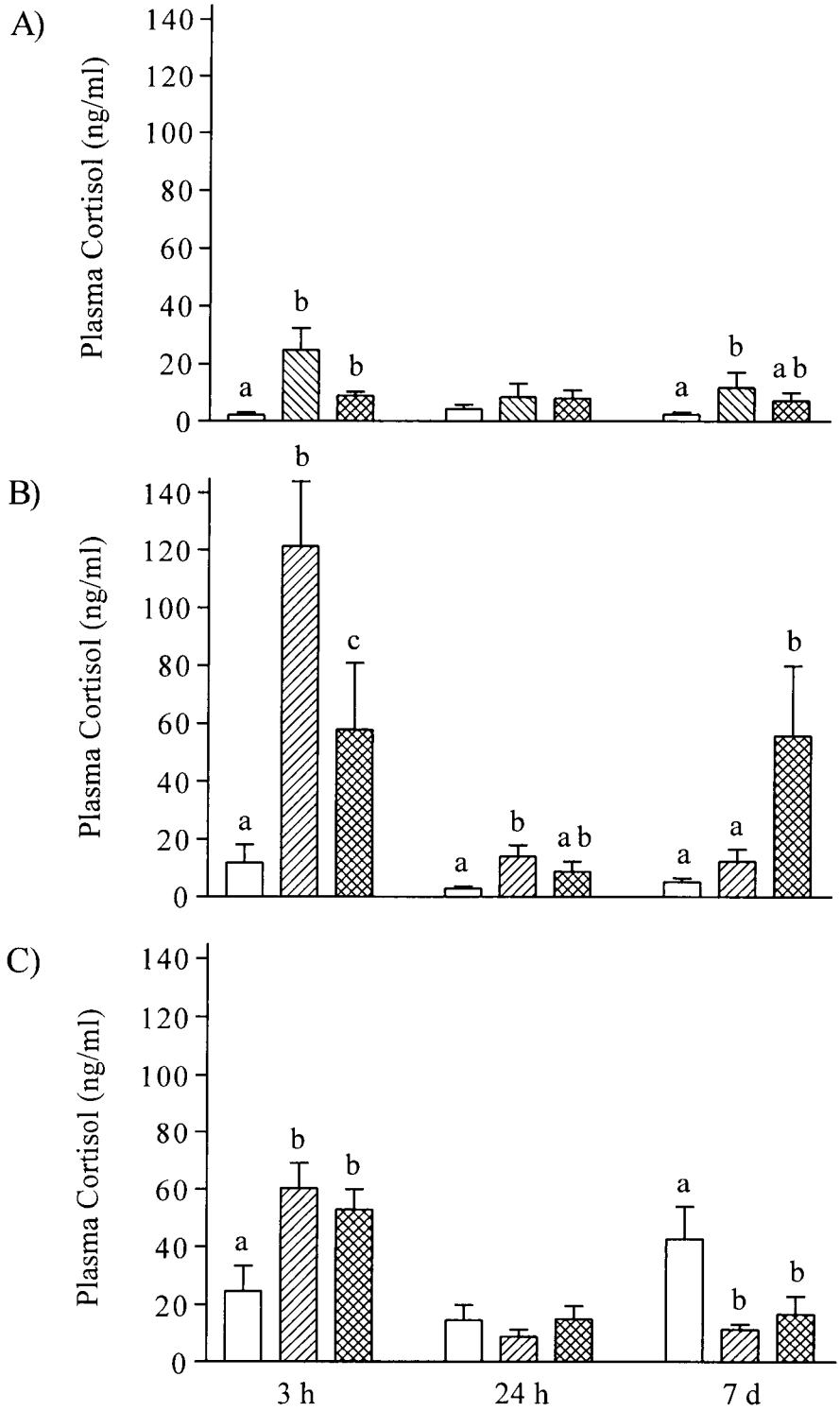

Figure 4. Mean (+ SE) plasma cortisol concentrations in juvenile spring chinook salmon exposed to a 2-s, 300-V, 50-Hz pulsed DC electroshock (▨), 30-s handling stress (▩), or no stress (□) from immune experiments I (A), II (B), and III (C). Means represent data from two replicates that did not differ statistically and were pooled. Results are given for three times after stress. Means within a time period without letters in common differ significantly.

Plasma glucose levels differed by treatment or sample time in immune experiments I (GLM F-test on ln transformed data, $P = 0.0001$) and III (GLM F-test, $P = 0.0001$) (Figures 5 A and C). Plasma glucose concentrations did not differ by treatment or sample time in immune experiment II (GLM F-test $P = 0.59$) (Figure 5 B). Glucose concentrations in electroshocked fish from immune experiment I were significantly higher than control and handled fish 3 h ($P = 0.0001$ and $P = 0.029$, respectively) and 24 h post stress ($P = 0.0007$ and $P = 0.012$, respectively). Handled fish were also significantly higher than controls at 3 h ($P = 0.0008$), but had returned to resting levels by 24 h after stress (Figure 5 A). Glucose levels in both electroshock and handling groups from immune experiment III were significantly higher than controls 3 h after stress ($P \leq 0.0003$). Concentrations in handled fish were elevated above controls and electroshocked fish ($P \leq 0.008$) at 24 h (Figure 5 C). There was suggestive, but inconclusive, evidence 7 d post stress that glucose levels in the electroshock group were lower than controls ($P = 0.051$). A second analysis after removal of an outlier from the control group, however, showed no indication of differences between the two groups ($P = 0.217$).

In vitro experiments

We found no difference in respiratory burst activity between leukocyte suspensions exposed to electroshock *in vitro* compared to controls in *in vitro* experiment I (Paired t-test, $P = 0.722$) (Figure 6 A). We did find convincing evidence

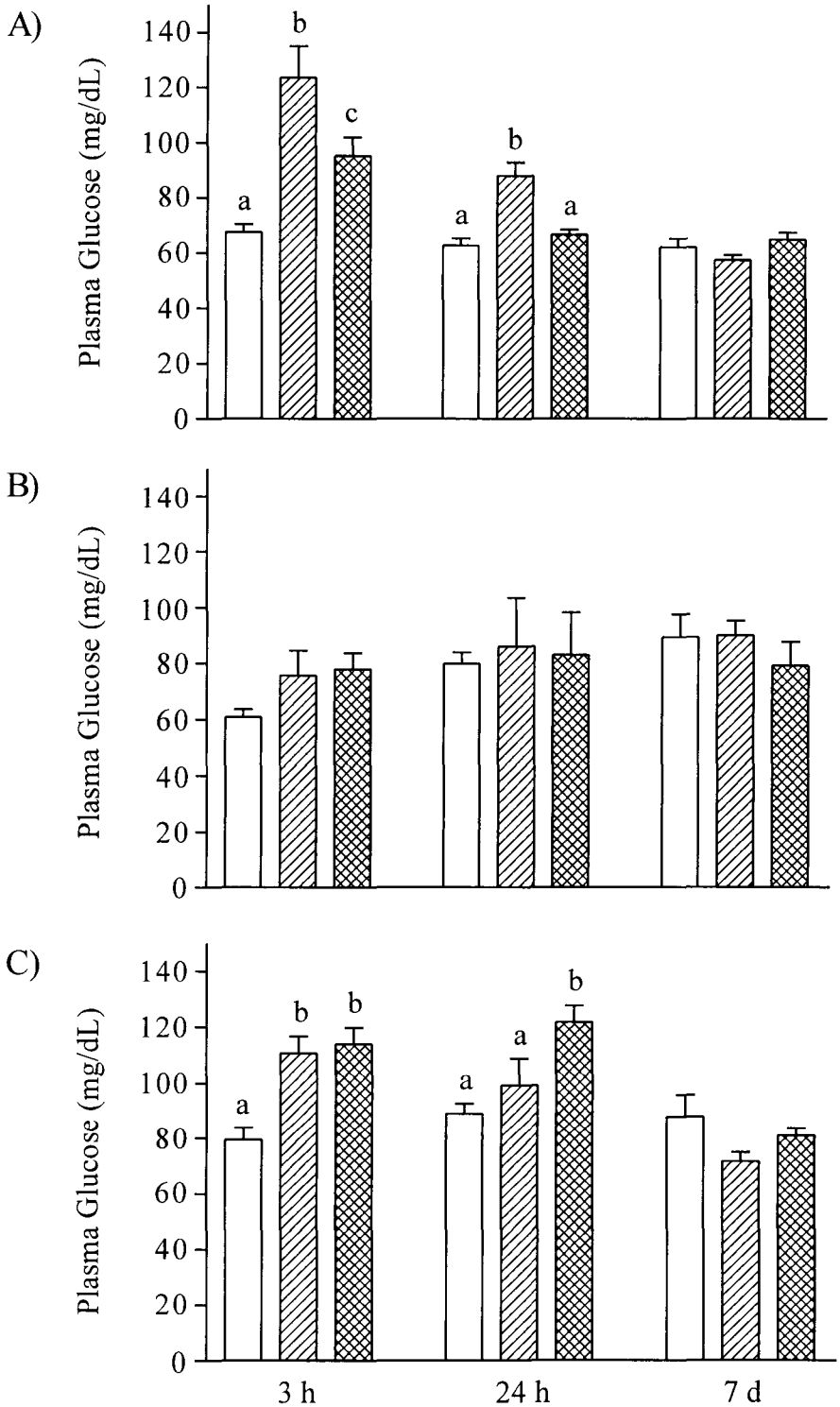

Figure 5. Mean (+ SE) plasma glucose concentrations in juvenile spring chinook salmon exposed to a 2-s, 300-V, 50-Hz pulsed DC electroshock (▨), 30-s handling stress (▩), or no stress (▭) from immune experiments I (A), II (B), and III (C). Means represent data from two replicates that did not differ statistically and were pooled. Results are given for three times after stress. Means within a time period without letters in common differ significantly.

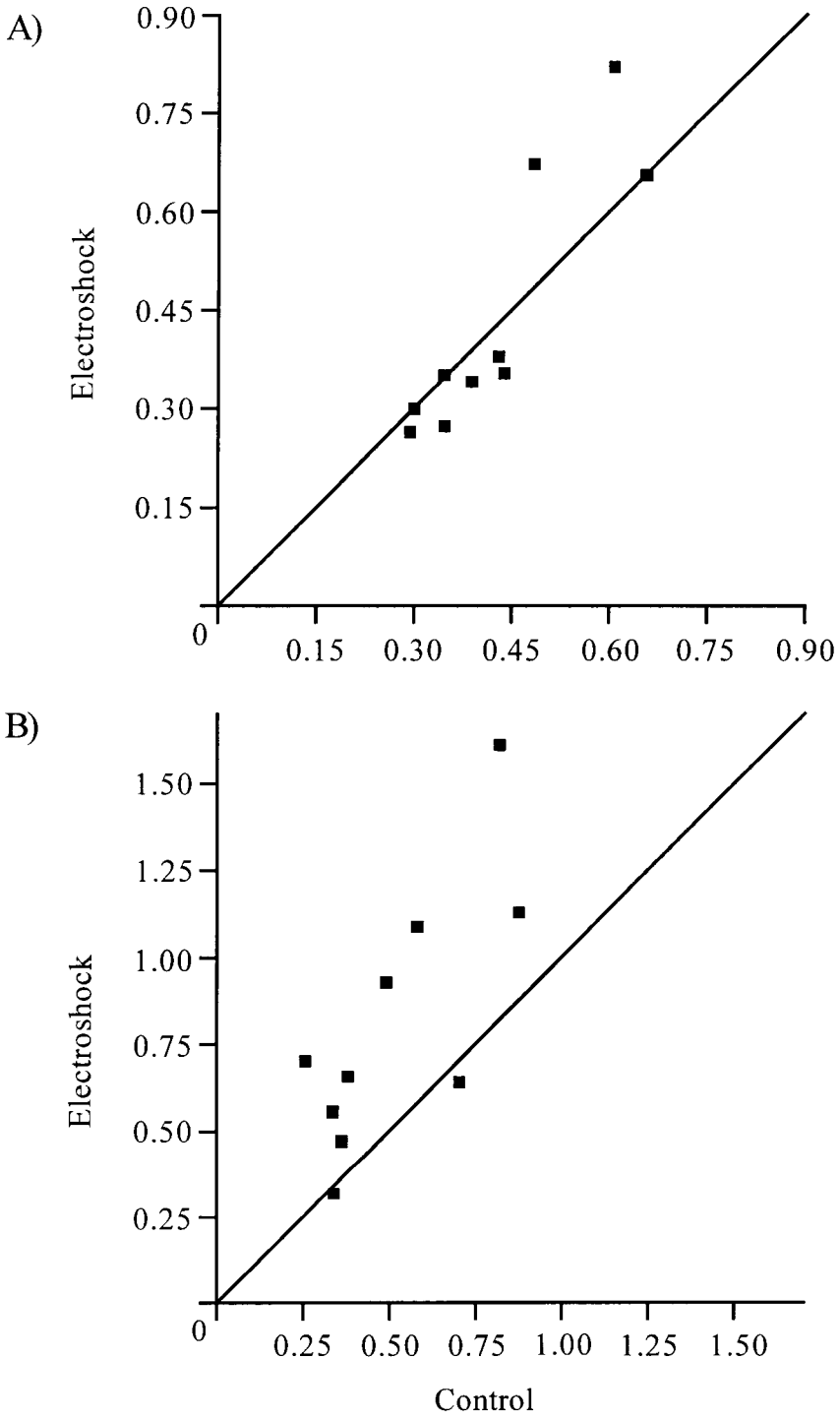

Figure 6. Difference in repiratory burst activity (nmol NBT reduced / 10^6 leukocytes) between control and treatment head kidney leukocyte suspensions. Treatment consisted of an *in vitro* 2-s, 300-V, 50-Hz pulsed DC electroshock. Each fish from *in vitro* experiments I (A) and II (B) served as its own control thus each point represents the response of control vs. treatment leukocyte suspensions from an individual fish. The diagonal represents where responses are equal.

from *in vitro* experiment II that respiratory burst activity was significantly higher in the electroshock group compared to controls (Paired t-test, $P = 0.0057$) (Figure 6 B). There was no evidence to indicate that the ability of leukocytes to produce APC after *in vitro* exposure to a pulsed DC electroshock was different from controls from *in vitro* experiments I or II (Paired t-test, $P \geq 0.61$) (Figures 7 A and 7 B).

Disease challenges

In disease challenge I, aside from one exception, no differences were detected in triplicate tanks (GLM F-test, $P > 0.05$). Data from replicates that were not different were pooled for further analysis. One tank of handled fish sampled 2 weeks post-stress was significantly different from the two other replicates (GLM F-test, $P = 0.0001$). This replicate was included in the final analysis as a separate treatment. We found strong evidence that mean OD values varied by treatment or sample time in disease challenge I (GLM F-test, $P = 0.0001$). Mean OD values of controls did not differ throughout the experiment. The mean OD of electroshocked fish at 2 weeks was higher than controls and handled fish, but lower than one tank of handled fish ($P \leq 0.027$) (Figure 8). The electroshocked fish, however, were not different than baseline controls (week 0) ($P = 0.3$). The mean OD of one tank of handled fish was significantly higher than the week-2 controls ($P = 0.0001$) (Figure 8). The electroshock group from week 4 was not different from controls at week 0 or week 4 ($P > 0.7$) (Figure 8).

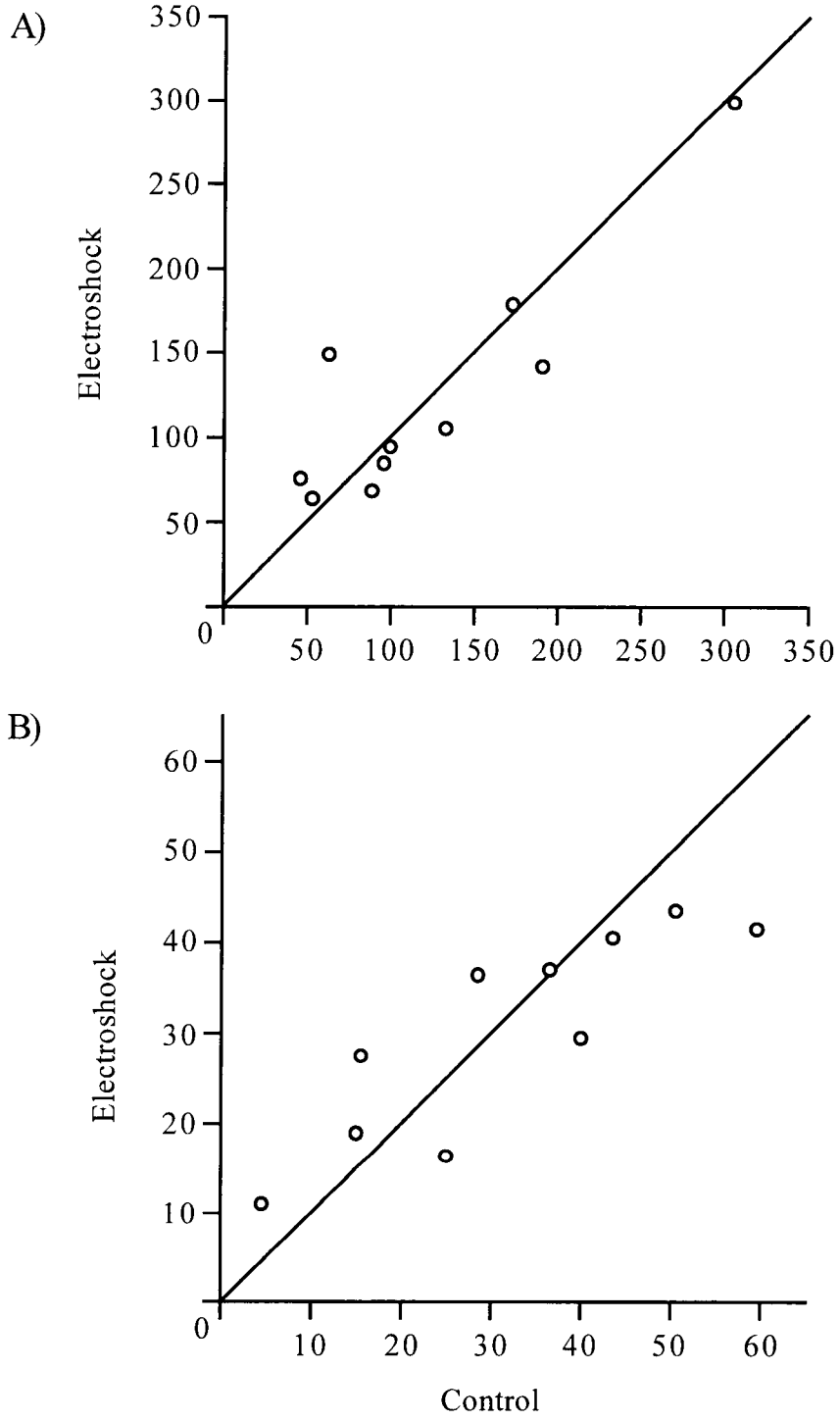

Figure 7. Difference in the number of antibody producing cells generated / 10^6 leukocytes between control and treatment head kidney leukocyte suspensions. Treatment consisted of an *in vitro* 2-s, 300-V, 50-Hz pulsed DC electroshock. Each fish from *in vitro* experiments I (A) and II (B) served as its own control thus each point represents the response of control vs. treatment leukocyte suspensions from an individual fish. The diagonal represents where responses are equal.

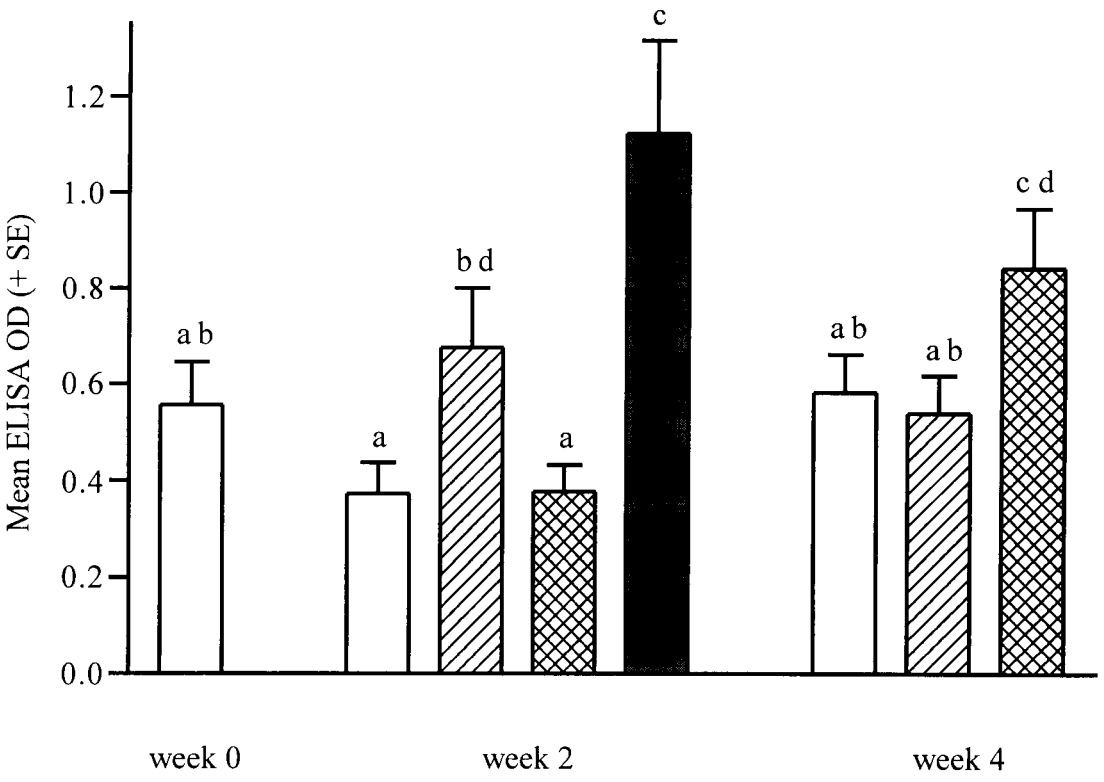

Figure 8. The effects of a 2-s, 300-V, 50-Hz pulsed-DC electroshock (▨) or 30-s handling stress (▩) on the progression of *Renibacterium salmoninarum* (RS) infection compared to unstressed controls (□). Disease challenge I results are presented as the mean ELISA OD (+ SE) for each treatment. Each bar represents pooled data from three replicates that do not differ significantly except handled fish from week 2. Two weeks after treatment a single replicate of handled fish (■) differed significantly from the other week 2 replicates of handled fish and was included as a separate treatment. The remaining week 2 handled replicates did not differ and were pooled. Treatments with no letters in common differ signifcantly.

In disease challenge II no differences in mean OD were found in replicates for positive controls or either treatment (Wilcoxon rank-sum test, $P > 0.05$), so these data were pooled for further analysis. One negative control tank was removed from the analysis because it became infected with RS. The infection resulted in low level mortality that began approximately 12 weeks after the start of the experiment and significantly different mean OD values between the negative control tanks from four of nine sample times ($P \leq 0.05$).

Prior to challenge with RS mean ELISA ODs did not differ by treatment. Mean ELISA ODs differed by treatment from weeks 2 through 12 after infection with RS (Kruskal-Wallis test, $P < 0.0002$). All RS-challenged groups had significantly higher mean ODs than the negative controls over the same period ($P < 0.004$). Mean ODs increased in all RS-challenged groups through week 6 with positive controls significantly lower than both the electroshock and handling groups 2 and 4 weeks after challenge ($P < 0.007$) (Figure 9). The mean ODs of electroshocked, handled and positive control fish did not differ at 6 weeks. Eight weeks after challenge the mean ODs of the electroshock and handling groups did not differ ($P = 0.14$) and were significantly higher than the positive controls ($P \leq 0.045$). No differences were detected between RS treatments 10 weeks after challenge ($P > 0.36$) and all had mean ODs over 1.0, the cutoff for high level infections (Figure 9). The highest mean OD was measured in the positive control group 12 weeks after challenge, this was significantly higher than the electroshock and handling groups ($P \leq 0.032$) which were

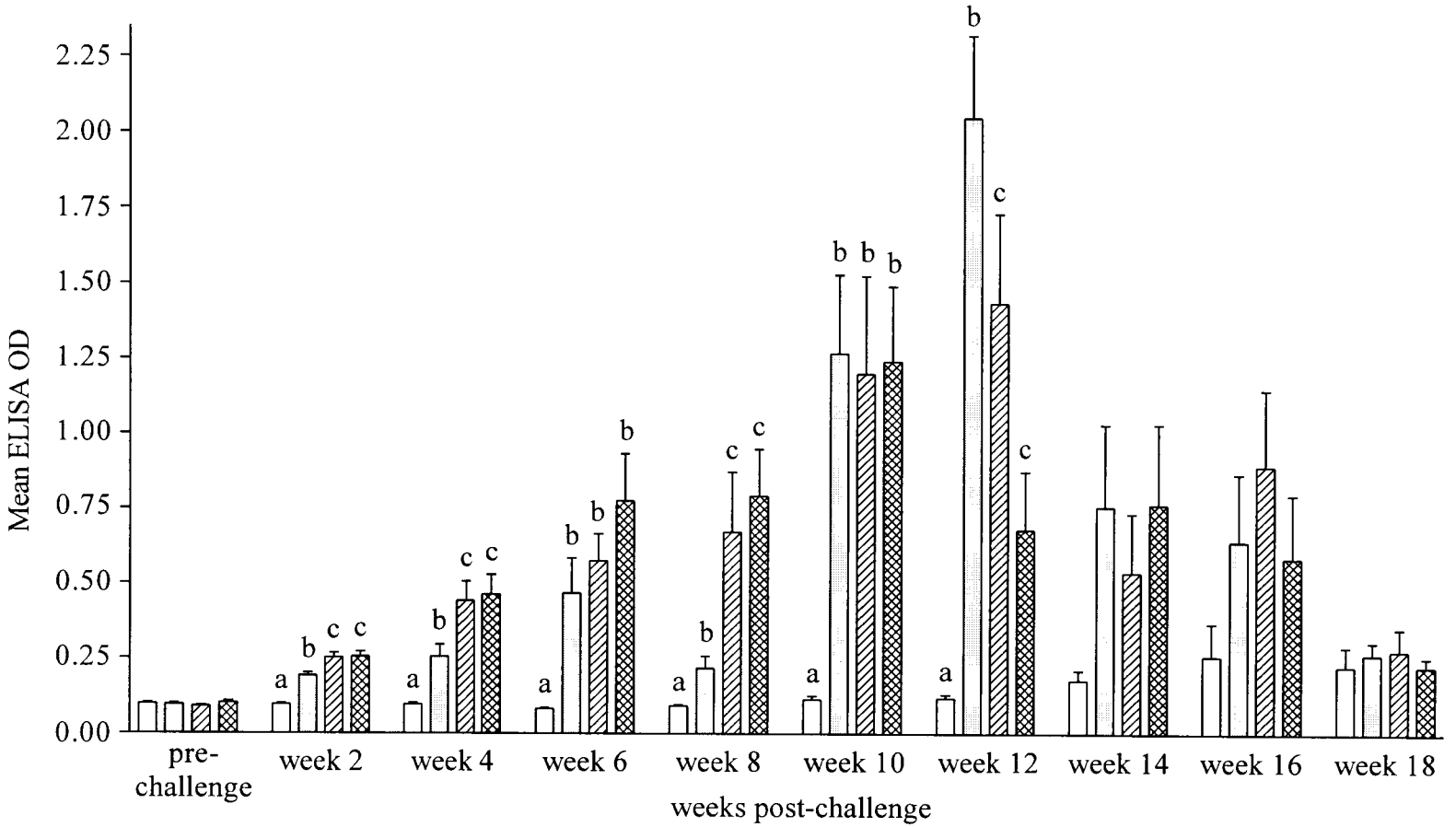

Figure 9. Mean (+ SE) ELISA OD of juvenile spring chinook salmon immediately prior to (pre-challenge) and at two week intervals after challenge with *Renibacterium salmoninarum* (RS). Negative controls (▭) were challenged with sterile saline while positive controls (▭) and other treatments were challenged with RS. Approxomately 7 weeks after challenge, fish were exposed to a 2-s, 300-V, 50-Hz pulsed DC electroshock (▨), 30-s handling stress (▩), or no stress (controls). Means represent data from two replicates that did not differ statistically and were pooled except for the negative controls which represent data from a single tank. Means within a time period without letters in common differ significantly.

not different ($P = 0.11$). Mean ELISA ODs did not differ between treatments 14, 16, or 18 weeks after challenge (Kruskal-Wallis test, $P > 0.12$) (Figure 9).

Neither plasma cortisol or glucose concentrations varied by replicate in disease challenge II (t-test, $P > 0.05$) so data were pooled for further analysis. Plasma cortisol levels varied by treatment at 3 h and 24 h after stress (GLM F-test on ln transformed data, $P \leq 0.02$). Electroshocked and handled fish were significantly higher than positive controls 3 h after treatment ($P \leq 0.0005$). The electroshocked fish were still higher than positive controls 24 h after exposure ($P = 0.028$). Cortisol levels in handled fish were not different from electroshocked fish or the positive controls at this time ($P \geq 0.12$). Electroshocked fish did not differ from the positive controls 72 h after stress ($P = 0.16$). Plasma glucose levels differed by treatment 3 h after stress (GLM F-test, $P = 0.0003$). Electroshocked and handled fish were significantly higher than positive controls 3 h after treatment ($P \leq 0.004$). No differences were detected between treatments at 24 h or 72 h after stress (GLM F-test, $P \geq 0.12$).

The number of mortalities varied within and among treatments (Figure 10). Since samples were collected from each tank at two week intervals it was not possible to determine the percentage mortality. The mean time to death of fish that died did not differ in duplicate tanks for any treatment (t-test, $P > 0.17$) so the data were pooled for further analysis. The mean time to death of fish that died was 5 d earlier for electroshocked fish and 2.5 d earlier for handled fish compared to the positive controls. There was inconclusive evidence that mean time to death of fish that died

Figure 10. Disease challenge II results presented as cumulative mortalities of juvenile spring chinook salmon after experimental infection with *Renibacterium salmoninarum* (RS). Negative controls were challenged with sterile saline while positive controls and other treatments were challenged with RS. Approximately 7 weeks after infection, fish were exposed to a 2-s, 300-V, 50-Hz pulsed-DC electroshock, 30-s handling stress, or no stress (controls). Each curve represents data from a separate tank.

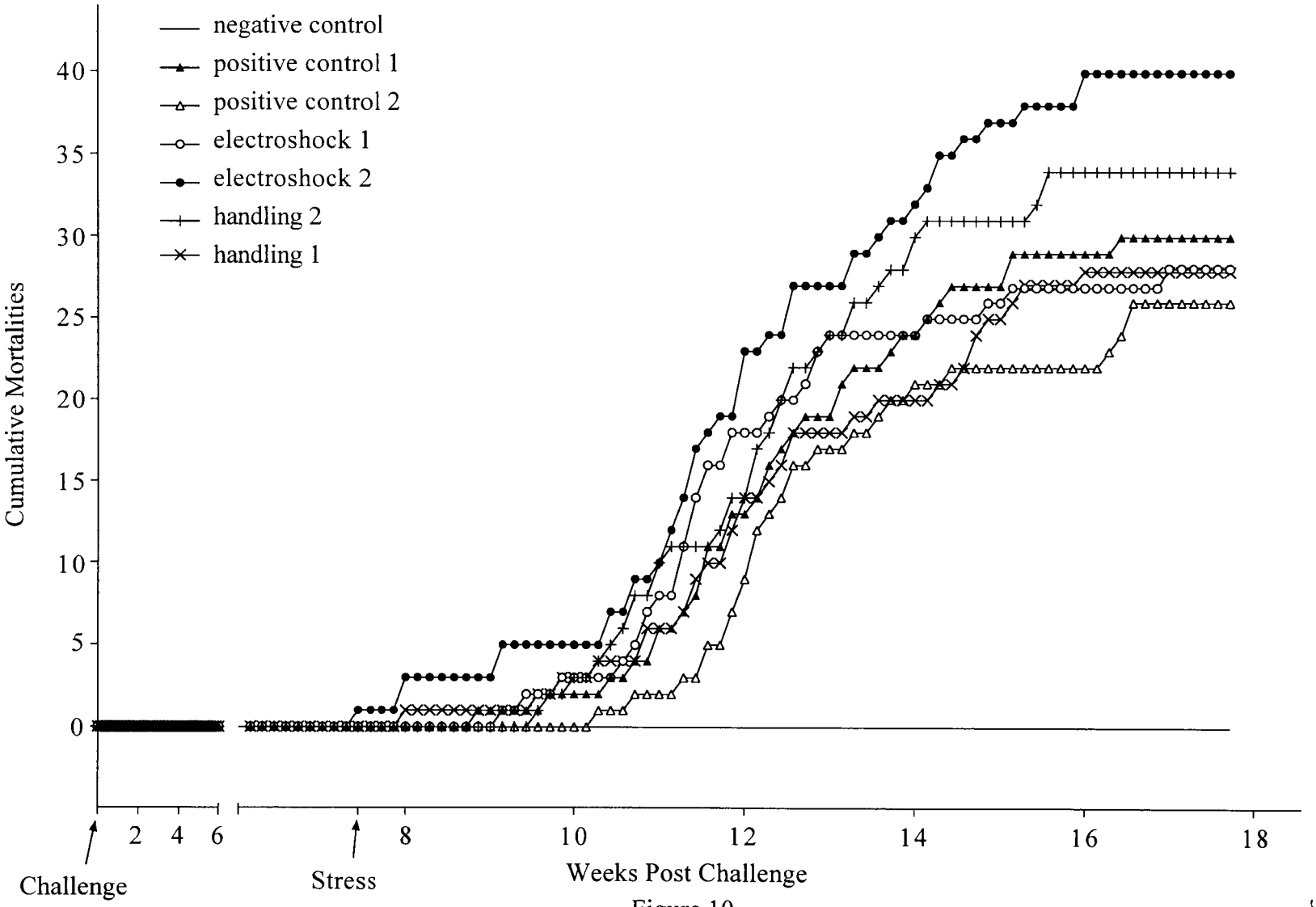
negative control
positive control 1
positive control 2
electroshock 1
electroshock 2
handling 2
handling 1
Cumulative Mortalities
0
5
10
15
20
25
30
35
40
2
4
6
8
10
12
14
16
18
Challenge
Stress
Weeks Post Challenge

Figure 10.

differed significantly by treatment (GLM F-test, P = 0.08). Multiple comparisons based on this analysis indicated the difference between electroshocked and positive control fish was significant (P = 0.025).

Discussion

Electroshock may temporarily suppress the specific immune response in juvenile chinook salmon. In two experiments the ability to generate antibody producing cells was reduced 3 h after exposure to electroshock with recovery to control levels seen within 24 h (Figures 3 A and B). The responses of electroshocked fish were similar to those of fish exposed to an acute handling stress. These results are similar to those found in the literature for juvenile chinook salmon subjected to an acute stress (Maule et al. 1989). In a third experiment, the only difference detected was 7 d after treatment when the numbers of APC produced by electroshocked fish were significantly lower than controls and handled fish (Figure 3 C). Maule et al. (1989) found the production of APC in juvenile chinook salmon to be significantly reduced 7 d after a handling stress. In our experiment however, the fish subjected to a handling stress were not different from controls at day 7 suggesting that electroshock may affect the specific immune response differently than a handling stress 7 d after treatment. We view this result with some skepticism since this effect was only observed in one of two trials with APC results at 7 d. The lack of the expected APC suppression at 3 h in either stress treatment also suggests that the fish in this experiment were in some way different from fish in the other trials or were influenced by some unknown factor. Further research is necessary to confirm that an electroshock can suppress APC production 7 d after exposure. Another caveat is that we used

leukocytes from a single source, the anterior kidney. Maule and Schreck (1990) found that an acute stress temporarily alters the number of leukocytes in the blood and immune system tissues. Given the dynamic nature of leukocyte populations after stress, our results may have been different if the responses of splenic, thymic, or circulating leukocytes had been monitored. The ability to generate APC in control fish varied widely between sample times and experiments. We believe the wide range of values observed was due to large amounts of inter-assy variability similar to that reported by other researchers (Maule et al. 1987; Tripp et al. 1987; Maule et al. 1989).

Our results indicate that skin mucous lysozyme levels in juvenile spring chinook salmon were not affected by exposure to electroshock (Figure 1). Plasma lysozyme levels have been reported to be elevated in rainbow trout after a short handling stress (Möck and Peters 1990; Demers and Bayne 1997) and reduced after longer stress treatments (Möck and Peters 1990). There are a number of possible reasons why our results differ from those reported in the literature. Mucous lysozyme levels may have been altered by electroshock, but recovered within 3 h of exposure. Another possibility is that the electroshock was not of a sufficient duration or severity to elicit a response. Responses may also vary by species given that baseline lysozyme concentrations differ by species (Grinde et al. 1988; Lie et al. 1989). Blood and mucous lysozyme may originate from different sources which could respond differently to stress. Lysozyme is found in neutrophils and macrophages (Murray and Fletcher 1976) and is thought to be produced by these cells (Lie et al. 1989). Intestinal

eosinophilic granule cells in Atlantic salmon also contain lysozyme in their secretory granules (Sveinbjørnsson et al. 1996), which suggests localized sources of lysozyme production.

Exposure to an electroshock had no consistent effect on phagocyte respiratory burst activity. No differences were detected between electroshocked fish and controls at any time in the first experiment (Figure 2 A). The only difference in a second trial was that respiratory burst activity in electroshocked fish was significantly higher than control and handled fish at 7 d (Figure 2 B). However, we also found that controls at 7 d were significantly higher than the 3 h controls. The lack of consistency among control fish makes it difficult to interpret the elevation seen in electroshocked fish 7 d after treatment. The amount of variability in respiratory burst activity we observed in both trials indicates either large amounts of natural variation or that activity levels were influenced by some unknown factor.

We chose to examine indirect and direct mechanisms to determine the source of any alteration in immune function. The most obvious indirect mechanism would be via the stress response. Stress can alter the responses of a number of 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). It is important to remember that despite temporary negative effects, the response to acute stress is considered adaptive (Ottaviani and Franceschi 1996). The stress response is a key component of Selye's General Adaptation Syndrome, by which an organism is

able to divert resources from non-protective physiological functions to the immediate needs of fight or flight (Selye 1976).

We found that cortisol levels in electroshocked fish were significantly higher than controls 3 h after treatment indicating that a stress response had been induced. We believe that the low magnitude of cortisol responses seen after stress in immune experiment I (Figure 4 A) was due to quick recovery rather than little or no response to stress. Plasma glucose results from this experiment support our contention (Figure 5 A). Recovery to control levels of cortisol occurred in most cases within 24 h (Figure 4). In the second experiment cortisol concentrations in electroshocked fish were higher than controls, but were not different from handled fish at 24 h indicating these fish were near recovery. The timing and magnitude of the stress response in electroshocked fish was similar to that of handled fish. Results were more variable at 7 d, but generally indicated electroshocked fish were not stressed 7 d after exposure. In immune experiment I, mean cortisol in the electroshock group was significantly higher than controls at 7 d, but was not different than handled fish. The change from levels at 24 h was slight and not considered to be biologically significant (Figure 4 A). Electroshocked fish did not differ from control fish at 7 d in the second experiment (Figure 4 B). In immune experiment III control fish had significantly higher cortisol levels than electroshocked fish 7 d after stress (Figure 4 C). However, the lack of differences between electroshocked fish at 7 d as compared to electroshocked fish or controls at 24 h indicate these fish were not stressed.

Results concerning plasma glucose also indicate that electroshock induces a stress response similar in magnitude and timing to that caused by a handling stress. In immune experiments I and III circulating glucose concentrations in fish from the stress treatments were significantly higher than controls 3 h after stress with recovery seen at either 24 h or 7 d (Figures 5 A and C). The significant increase seen at 3 h in immune experiment I indicates these fish were indeed stressed, supporting our contention that plasma cortisol levels recovered quickly from stress. It is unclear why glucose concentrations in fish from the second experiment from did not respond to stress (Figure 5 B).

We found some evidence that an electroshock has a direct effect on respiratory burst activity, but not on the generation of APC. Respiratory burst activity was enhanced *in vitro* immediately after electroshock in one of two trials (Figure 6 B). If this is also the case *in vivo*, the effect is short lived given the lack of differences seen 3 h after electroshock in the immune function experiments. An enhancement *in vivo* would contradict the suppression of respiratory burst activity after stress reported in the literature (Angelidis et al. 1987; Thompson et al. 1993). We view these results with caution given that enhancement was seen in only one of two trials. Further research is necessary to confirm this effect.

The ability of leukocyte suspensions to generate APC was not affected immediately after an *in vitro* electroshock (Figure 7). A caveat to this finding is the possibility of effects 3 h after exposure that were not detected due to unavoidable

constraints on the timing of this experiment. The lack of a direct effect of electroshock on the generation of APC would suggest that immune suppression occurs via an indirect route. This finding in conjunction with the responses of the stress indicators leads to the conclusion that suppression of APC generation 3 h after electroshock was due to elevated plasma cortisol concentrations in response to stress. This is consistent with the mechanism of immune suppression reported by other researchers (Maule et al. 1987; Tripp et al. 1987). Tripp et al. 1987 found that immune suppression due to cortisol was dose dependent. In contrast, we found no significant correlations between cortisol concentrations and the ability to generate APC in any of our trials. Cortisol interferes with the specific immune response by temporarily suppressing the proliferation of APC from precursor cells (Kaattari and Tripp 1987) while stress induces changes in the number of leukocytes in immune tissues (Maule and Schreck 1990). Our results do not indicate whether suppression of the specific immune response was due to reduced immune cell activity or a change in the number of head kidney leukocytes. It is also unclear from our results what mechanism may be responsible for the suppression of APC seen 7 d after electroshock.

We found some evidence that electroshock can affect the progression of disease. In the first experiment with disease challenge, the fish exposed to electroshock had a mean ELISA OD that was significantly higher than control or handled fish 2 weeks after stress (Figure 8). These results would seem to suggest that electroshock temporarily alters the progression of an RS infection in juvenile chinook

salmon. However, we also found the mean OD of electroshocked fish was not different from that of the baseline controls (week 0). Results from 4 weeks after stress showed that the mean OD of electroshocked fish was not different from the week 4 or week 0 controls (Figure 8). The lack of differences from baseline OD levels 2 and 4 weeks after treatment demonstrate that electroshock did not affect the progression of infection.

Results from the second disease challenge indicated that exposure to electroshock had some effect on the progression of an RS infection, suggesting that time to death may be accelerated. The stress response of infected fish was normal. Electroshocked and handled fish had significantly elevated plasma cortisol and glucose levels 3 h after stress. Recovery to control levels occurred between 24 and 72 h after stress. We did find that the mean OD of the electroshocked fish was significantly higher than the infected, unstressed positive controls 4 d after treatment (8 weeks post-challenge) (Figure 9). The mean OD at week 8, however, was only slightly higher than pre-electroshock levels (week 6) while the mean OD of positive control fish dropped over the same period. These changes suggest that the difference at week 8 was due to a random change in positive control levels rather than some effect of the electroshock. The lack of differences between RS-infected treatments 10 weeks post-challenge support this conclusion (Figure 9). Further proof of the lack of influence of electroshock on the progression of RS was found at week 12 when the mean OD of the positive controls was significantly higher than electroshocked fish (Figure 9). Mean

ODs dropped from peak levels and did not differ by treatment, including the negative controls, from week 14 through the end of the challenge (Figure 9). These results indicate that electroshock had no long-term effect on the progression of an RS infection

There was no clear effect of electroshock on cumulative mortality levels in RS infected fish. The highest number of mortalities were in one electroshock replicate, followed by a replicate of handled fish. On the other hand, the replicates for each of these treatments had equal numbers of mortalities. The mortality level of these replicates was almost exactly intermediate of the two positive control replicates (Figure 10). Although mortality levels varied, the similar shape of each mortality curve indicates each replicate responded to the infection in a like manner. We did find some evidence that the mean time to death of fish that died was significantly shorter in electroshocked fish than in the positive controls. The mean time to death of electroshocked fish was 5 d earlier than positive controls, with the handled fish intermediate of these treatments. The biological significance of this difference is unclear given that cumulative mortality of one replicate of electroshocked fish was intermediate of the two positive controls. Disease resistance in salmonids is affected by stress (Maule et al. 1989), but research into the effects of stress on sick fish is lacking. Our research shows that the electroshock did induce a stress response and that the progression of RS infection in terms of severity or the numbers of mortalities

was not affected. It is not clear from this work what mechanism might be responsible for the acceleration in time to death.

Our research indicates that exposure to an electroshock briefly suppresses the specific immune response 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 lead us to conclude that electrofishing is a safe procedure in regards to immunity and disease. A caveat of this research is that only one set of electroshock settings were tested. Mortality and injury rates vary under different exposure conditions and it is possible that changes in voltage, current type, pulse frequency, temperature, or a number of other factors might yield different results.

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