RECLAMATION

Managing Water in the West

Tracy Series Volume 55

Effects of Fin-Clipping for DNA Sampling on Tissue Damage, Physiological Stress, Swimming, and Survival of Juvenile Chinook Salmon

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14. ABSTRACT

Collection of tissue samples from Chinook Salmon (*Oncorhynchus tshawytscha*) captured at federal and state fish salvage facilities in California's southern Sacramento-San Joaquin Delta for genetic tissue analyses are important for species management, as this data provides an understanding of temporal variation in Evolutionarily Significant Unit (ESU)-specific salmon migration and abundance. However, means to acquire tissues (i.e., caudal fin-clipping) should not compromise survival, as this would conflict with conservation efforts. An on-site study at the Bureau of Reclamation's Tracy Fish Collection Facility (Byron, CA) was completed to quantify effects of handling and fin-clipping procedures on physiological stress, external tissue damage, burst swimming performance, and survival of juvenile Chinook Salmon during three unique sample periods (April, May, and June 2010). In addition, effects of water treatment additives (i.e., NaCl and commercially available slime coat) applied post tissue sampling were evaluated. Survival of Chinook Salmon, regardless of treatment condition, throughout the 168-h post-treatment assessment period was high (> 94%). However, effects of handling and clipping on survival of juvenile Chinook Salmon were influenced by post-treatment holding environment and varied as a function of testing period. Experimental results indicate there was no difference in physiological stress of salmon exposed to handling only or handling and clipping, suggesting fin-clipping alone provided no significant additional stress, and netting, anaesthetization, and handling fish is probably the most stressful component of the fin-clipping process. In addition, within month of testing and sample period (0, 2, or 168 h) clipping did not contribute to an increase in tissue damage compared to handling alone, and the use of treated water did not minimize tissue damage.

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by

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Collection of tissue samples from Chinook Salmon (*Oncorhynchus tshawytscha*) captured at federal and state fish salvage facilities in California's southern Sacramento-San Joaquin Delta for genetic tissue analyses are important for species management, as this data provides an understanding of temporal variation in Evolutionarily Significant Unit (ESU)-specific salmon migration and abundance. However, means to acquire tissues (i.e., caudal fin-clipping) should not compromise survival, as this would conflict with conservation efforts.

An on-site study at the Bureau of Reclamation's Tracy Fish Collection Facility (Byron, CA) was completed to quantify effects of handling and fin-clipping procedures on physiological stress, external tissue damage, burst swimming performance, and survival of juvenile Chinook Salmon during three unique sample periods (April, May, and June 2010). In addition, effects of water treatment additives (i.e., NaCl and commercially available slime coat) applied post tissue sampling were evaluated.

Survival of Chinook Salmon, regardless of treatment condition, throughout the 168-h post-treatment assessment period was high (> 94%). However, effects of handling and clipping on survival of juvenile Chinook Salmon were influenced by post-treatment holding environment and varied as a function of testing period. Experimental results indicate there was no difference in physiological stress of salmon exposed to handling only or handling and clipping, suggesting fin-clipping alone provided no significant additional stress, and netting, anaesthetization, and handling fish is probably the most stressful component of the fin-clipping process. In addition, within month of testing and sample period (0, 2, or 168 h) clipping did not contribute to an increase in tissue damage compared to handling alone, and the use of treated water did not minimize tissue damage.

Introduction

Abundance and distribution of Chinook Salmon (*Oncorhynchus tshawytscha*) in California's Central Valley has declined severely from historic levels (Yoshiyama et al. 1998; Yoshiyama et al. 2000). In response, conservation efforts have been established in an attempt to preserve and restore populations (Hedgecock et al. 2001). Continued exploration of the population genetics of Central Valley, and other Pacific Coast, salmon is an important management tool, furthering such efforts (Bartley and Gall 1990; Waples 1995; Hedgecock et al. 2001). Genetic markers assist in defining Central Valley Chinook Salmon genetic diversity and structure, which lead to defining population structure and distinct Evolutionary Significant Units (ESU; Banks et al. 2000; Hedgecock et al. 2001). Classification of a particular salmon population as an ESU warrants consideration for listing under the U.S. Endangered Species Act, thereby furthering conservation efforts (Waples 1995). Also, genetic analyses permit an understanding of temporal variation in ESU-specific salmon migrations and abundance (Hedgecock et al. 2001). To acquire tissue samples for genetic analyses, excising a portion of a fish's fin is widely employed (Banks et al. 2000; Williamson and May 2005). Though genetic data are critical to conserve and manage endangered and threatened salmon (Waples et al. 1990; Waples 1994; Waples 1995), means to acquire tissue samples should not directly or indirectly compromise survival, thereby further compounding conservation efforts.

Published data on effects of fish fin-clipping is confounding. Certain data suggest fin-clipping can greatly reduce survival and hinder growth (Saunders and Allen 1967; Shetter 1967; Webber and Wahle 1969; Coble 1971; Nicola and Cordone 1973; O'Grady 1984; Bergstedt 1985; Hansen 1988), and similarly, extensive fin damage caused by tissue sampling may compromise survival (O'Grady 1984). Conversely, fin-clipping has been shown to have no effect on survival or growth (Armstrong 1947; Radcliffe 1950; Horak 1969; Gjerde and Refstie 1988; Conover and Sheehan 1999; Pratt and Fox 2002; Vander Haegen et al. 2005; Champagne et al. 2008). Handling and severing fins is reportedly stressful to fish (Sharpe et al. 1998; Barton et al. 2002), and provides a potential vector for bacterial infection (Elliot and Pascho 2001; Vander Haegen et al. 2005). Decreased survival of fish can result when physiological stress responses remain elevated and become debilitating, leaving fish vulnerable to predation or swimming challenges (Barton 2002; Portz 2007). Because reduction in surface area of fins as a result of fin-clipping could potentially reduce swimming capacity, and the ability to evade predators and compete to acquire resources, a few studies have examined the effects of fin-clipping on swimming velocity (Radcliffe 1950; Horak 1969; Champagne et al. 2008); however to our knowledge no studies involving burst swimming have been performed. Burst swimming is important in evading predators, catching prey, and danger avoidance (Portz 2007).

Juvenile Chinook Salmon fin-clipping, to acquire a tissue sample for DNA analyses, is a common practice at state and federal water project facilities in California's Central Valley to estimate timing, abundance, and proportion of different Chinook Salmon ESU's traversing the Sacramento-San Joaquin Delta (SSJD) in California's Central Valley. Genetic tissue sampling at the Central Valley water project facilities resulted in the handling and clipping of nearly 3,000 Chinook Salmon between 2009 and 2012 (Reyes Pers. Comm. 2013). Because of the abundance of juvenile salmon processed at these facilities, the primary objective of this research was to quantify effects of handling and fin-clipping on physiological stress, external tissue damage,

burst swimming performance, and survival. The secondary objective was to determine if water treatment additives (i.e., NaCl and commercial protective slime coating), commonly used during or following handling of fish (Harmon 2009), but not currently used during fin-clipping operations at the water project facilities, could reduce external tissue damage and physiological stress response, and improve survival of handled and clipped Chinook Salmon.

Methods

Fish Source and Care

Juvenile Sacramento River Chinook Salmon (*Oncorhynchus tshawytscha*) were truck-transported ~82 km, in March 2010, from California Department of Fish and Wildlife's Mokelumne River Hatchery (Clements, CA) to the Tracy Fish Collection Facility (TFCF; Byron, California; Bureau of Reclamation), where they were maintained in 757-L circular tanks and provided a mixture of temperature controlled and aerated well and SSJD water. Salmon were maintained under a natural photoperiod (37° 44' 23" N latitude) with natural and halogen light, and fed Silver Cup salmon feed (Nelson and Son, Inc., Murray, Utah) at 1.5–2% body weight per day prior to testing. At least two weeks prior to each experimental period, test fish were marked with colored microspheres on dorsal and anal fins with a high pressure photonic tagging gun (New West Technology, Arcata, California), permitting consolidation of fish during post treatment 168 h survival assessments. This marking procedure was employed because, when paired with a sufficient (> 1 week) post-marking recovery period, it presumably does not impair fish stress response (Sharpe et al. 1998; Hayes et al. 2000) or swimming performance (Sutphin et al. 2007).

Tracy Fish Collection Facility Standardized Genetics Tissue Collection Procedure

Central Valley water project facilities standardized operating procedure (SOP) for genetics tissue collection of Chinook Salmon was followed during testing. The following is a summarization of the SOP pertinent to the current experiment: (1) net salmon from fish count station (Figure 1) and place in ambient temperature SSJD water containing 50 ppm tricaine methanesulfonate (MS 222) for anaesthetization (< 10 fish and < 15 min exposure), (2) check each salmon for coded wire tag and only process non-coded wire tagged fish, (3) using iodine sterilized scissors and moistened hands, clip small sample (~ 2×4 mm, minimum of 1×1 mm sample) from upper or lower caudal lobe (Figure 2 and Figure 3), (4) transfer tissue sample to vial containing 95% ethanol, (5) measure and record fork length (mm), (6) transfer salmon to recovery aquarium containing aerated SSJD water and (7) permit full recovery from anesthesia before release. In addition to the SOP, a side profile picture was recorded for all clipped salmon.

Figure 1.—Tracy Fish Collection Facility (Byron, CA.; Bureau of Reclamation) Fish Diversion Worker using a fine-mesh dip net to remove juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) from the facilities Fish Count Station where fish are condensed and processed (identified, counted, and measured for length) following capture.

Figure 2.—Tracy Fish Collection Facility (Byron, CA.; Bureau of Reclamation) Fish Diversion Worker acquiring a juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) caudal fin-clip (INSET) for genetic analyses.

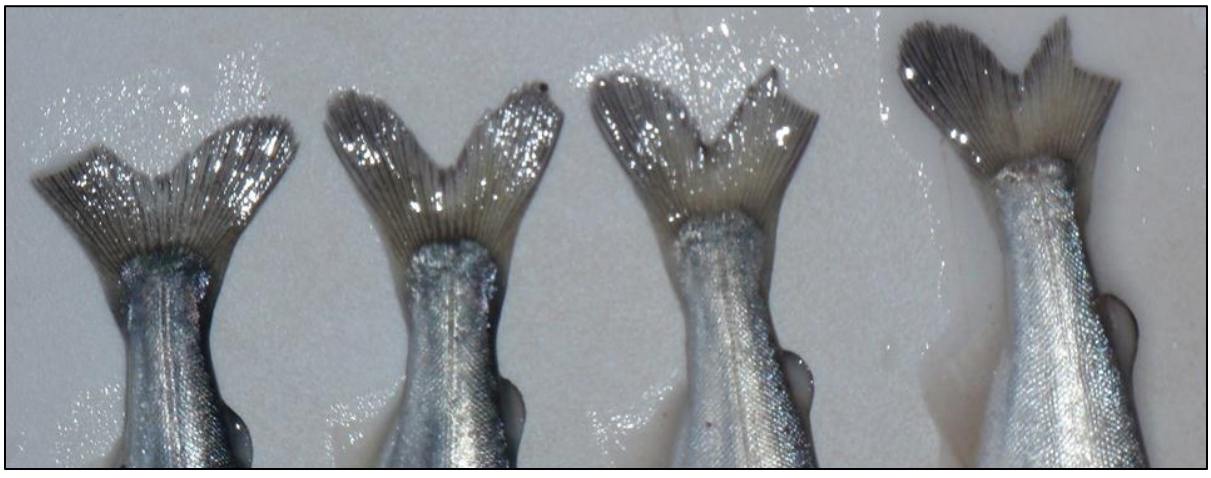

Figure 3.—Example of caudal lobe fin-clip sizes excised from juvenile Chinook Salmon (*Oncorhynchus tshawytscha)* during routine tissue sampling for genetic analyses at Bureau of Reclamation's the Tracy Fish Collection Facility (Byron, CA; Bureau of Reclamation).

Experimental Protocol and Treatment Groups

Experiments were conducted at the TFCF, allowing the use of the equipment employed during standard fin-clipping operations and the use of SSJD water during post-treatment evaluations, exposing fish to natural environmental factors including unique water quality and potential pathogens. Also, TFCF Fish Diversion Workers completed fin-clipping during testing, as would occur during standard DNA sampling under normal operations at both state and federal water project facilities. This eliminated potential bias of having a biologist, proficient at handling fish, complete fin-clipping. Experiments were conducted over three distinct periods when juvenile Chinook Salmon are typically encountered at the facilities: April, May, and June 2010 (http://www.dfg.ca.gov/delta/apps/salvage). Conducting experiments over a three-month period permitted comparison of temporal variation, but perhaps more importantly size and thermal effects, in salmon response to handling and fin-clipping.

For each replicate, fish were randomly selected from one of four 757-L holding tanks and immediately relocated to a covered black bucket using water-to-water transfer (modified 10-cm x 18-cm dip nets with 1.5-l plastic reservoir sewn into the cod-end), minimizing stress as a result of atmospheric exposure and net induced trauma (Barton and Iwama 1991; Sharpe et al. 1998). Handled and clipped fish were immediately exposed to standard tissue sampling protocol (e.g., anaesthetization, handling, and clipping) as described previously (see *TFCF Standardized Genetics Tissue Collection Procedure*). Whereas, handled, but not clipped, fish were exposed to the same sampling protocol, but were not provided a fin clip. Following fin-clipping (H/C) or handling (H), salmon were randomly isolated and one individual from each treatment condition (H or H/C) was immediately assessed for stress response (see *Physiological Stress Response*), external damage (see *External Tissue Damage*), and swimming performance (see *Fish Swimming Performance*).

The remaining fish from each treatment condition were transferred to one of four air equilibrated post-treatment holding aquariums (151 L): Handled and Clipped in SSJD Water (H/C ΔW), Handled and Clipped in Treated Water (H/C TW), Handled Only in SSJD Water (H ΔW), or Handled Only in Treated Water (H TW). Treated water (TW) used during testing consisted of ozonated and ultraviolet light sterilized SSJD water supplemented with NaCl (4 ppt) and a water conditioner (PolyAqua, Kordon LLC, Hayward, California). Treated water was used to determine if use of conditioned water for short-duration recovery, following fin-clipping, would minimize physiological stress, external tissue damage, and improve survival. Fish were exposed to these conditions for two hours, after which three fish from each tank were removed, and one individual from each treatment condition were again assessed for stress response and external damage. The remaining fish (18–24 fish/treatment) were transferred to a circular 190-L tank for evaluation of 168-h survival. Control fish (CONTROL) were handled as little as possible, fish were pulled directly from 757-L holding tanks and one each was immediately assessed for stress response, external damage, and swimming performance, while the remaining fish were immediately transferred into a 190-L tank for evaluation of 168-h survival. Treatment fish were exposed to one of two conditions: pure SSJD water or ozonated and ultraviolet sterilized SSJD water during the 168-h survival assessment period. One replicate group for each treatment condition was combined into a single tank during the 168-h survival assessment. During the 168-h post-treatment survival period, tanks were covered with shade cloth to promote quiescent conditions, and only disturbed once daily for feeding, water quality (°C, DO, and pH)

measurements, and to check for mortalities. Following 168-h, fish were removed from the survival tanks, and for each treatment one fish each was assessed for physiological stress response and external damage. All salmon were measured for length (mm total length) and wet weight (g) during testing. For each treatment, a single replicate was carried out simultaneously, and 12 replicates were completed for each time (month) × treatment combination.

Physiological Stress Response

Blood plasma cortisol, glucose and lactate, as well as hematocrit (% packed cell volume) levels are commonly measured to assess effects of stressors on fish (Bonga 1997; Barton 2002), and were used to measure effects of handling and fin-clipping on the physiological stress response of juvenile Chinook Salmon. To sample blood plasma constituents, test fish were quickly transferred to a bath containing a lethal dose of MS-222 (Argent Chemical Laboratories, Inc., Redmond, Washington; 200 mg/L), resulting in rapid (< 30s) immobilization. This anesthetic dose inhibits stress-related increases in plasma cortisol concentration in salmon (Barton et al. 1986; Barton 2000). Following immobilization, blood was immediately collected from a severed caudal peduncle in 40-µl heparinized microhematocrit capillary tubes. Blood samples were immediately centrifuged using a microhematocrit centrifuge (Clay-Adams Autocrit Ultra3) for 4 min at 12,000 x g to separate plasma from packed cells (Becton Dickinson Diagnostics, Sparks, Maryland). Hematocrit was measured immediately following centrifuging, and blood plasma from each fish was transferred into plastic cryogenic freezing vials and temporarily stored in a 10-L liquid-nitrogen dewar flask (-196˚C). Following each experimental period (month of testing), samples were shipped to Denver, CO where they were stored in a -80ºC freezer. Plasma cortisol concentrations were measured using a modified enzyme immunoassay (ELISA) at the University of California, Davis Endocrinology Lab, and plasma lactate and glucose levels were measured with a polarographic analyzer (YSI 2700 Select, Yellow Springs Incorporated, Yellow Springs, Ohio) in Reclamation's Fisheries and Wildlife Group's Fish Physiology Lab (Denver, CO).

External Tissue Damage

Scale loss, external tissue damage, and ulceration as a result of handling and fin-clipping was assessed using fluorescein (AK-Fluor®, Akorn, Inc., Decatur, Illinois; Figure 4), a nontoxic fluorescent dye that is used to rapidly to detect scale loss, tissue lesions, and ulcers by binding to breaks or tears in the epithelial barrier of soft tissue (Noga and Udomkusonsri 2002). To assess external tissue damage, fish were anesthetized in a MS-222 bath (40 mg/L) and transferred to a solution of 0.20 mg fluorescein/1 ml water for 5 min, then rinsed in three separate "clean" water baths for 2 min. Forceps clamped to the anal fin were used for fish transfers to minimize bodily tissue damage. Following rinsing, salmon were euthanized in a 200 mg/L MS-222 bath, and immediately examined for skin damage under an ultraviolet light (Model UVGL-58, Mineralight, Upland, California). Photographs were taken in complete darkness under ultraviolet light using a Nikon D-100 digital camera. Fish were placed on black background for capturing all images. The camera was affixed in a stationary position to allow a similar angle and distance of captured images across fish/treatments, and images were taken with flash off and a shutter speed of 2 seconds. Percentage of area damaged was determined by dividing the number of fluoresced pixels (damaged area) by the total number of pixels (entire salmon) from each of two

lateral views using software from VICON MOTION SYSTEMS, INC. (Centennial, CO). External tissue damage analyses were conducted through all post-treatment sample periods (0, 2, and 168 h) in May and June, but only at 0 and 2h in April.

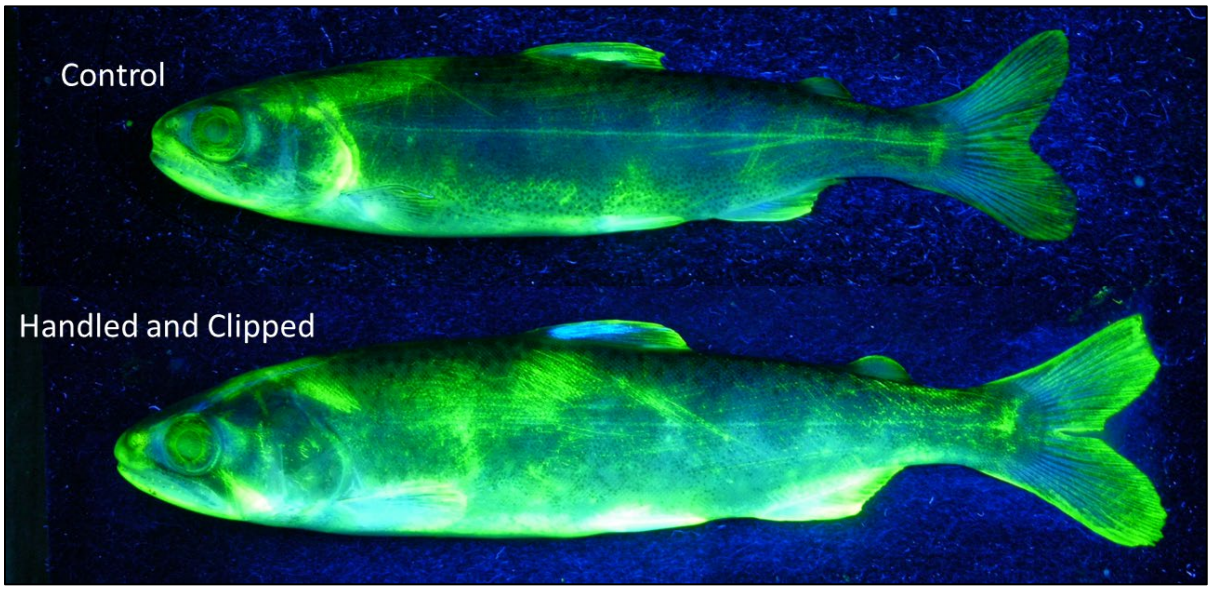

Figure 4.—Image of fluorescein dyed Chinook Salmon (*Oncorhynchus tshawytscha*) with minimal handling (Control; top image) and 2 hours following handling + clipping procedures employed at Bureau of Reclamation's Tracy Fish Collection Facility (Byron, CA) to acquire tissue samples for genetic analyses.

Fish Swimming Performance

Circular fish swimming flumes (24" long × 10" wide × 5" deep; Figure 5) were used to measure effects of handling and clipping on burst swimming performance of test fish. For each replicate, salmon were transferred to one of two swimming flumes, provided 30 s exposure to quiescent conditions (zero velocity), after which velocities were increased at a rate of ~ 5 cm/s until 80 cm/s was reached. A target velocity of 80 cm/s was selected because this is reportedly near the upper burst swimming speed of juvenile salmonids (Bainbridge 1960; Randall et al. 1987). Once the target velocity was achieved a stopwatch was used to measure time until the fish became fatigued, which was defined as complete impingement or three touches of the caudal fin on the downstream end of the test chamber within a 5 sec period, whichever occurred first. Once fatigue was observed, velocity in the flume was returned to 0 cm/s, and fish were removed. During the swimming experiment, fish that became impinged on the downstream end of the test chamber before 80 cm/s velocity was reached were deemed to have not participated in the experiment. Fish swimming flumes were calibrated daily before testing using a Marsh McBirney flow meter (Hach Company, Loveland, Colorado). Water temperature (°C) was measured at the end of each replicate, and dissolved oxygen (DO; mg/L) was monitored throughout testing using a YSI85 Multimeter (YSI Inc., Yellow Springs, Ohio). Water was changed in each swimming flume when DO levels were < 7.0 mg/L.

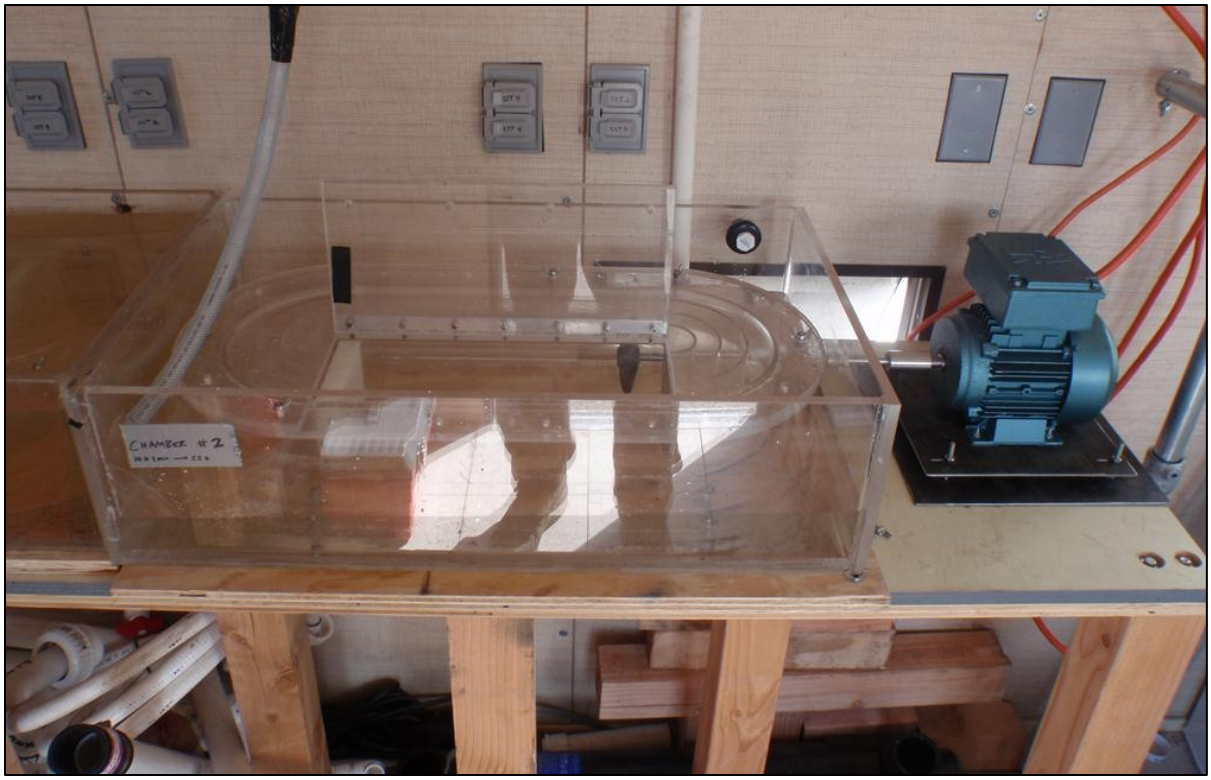

Figure 5.—Fish swimming flume (24” long × 10” wide × 5” deep) and external variable speed 120V/60Hz motor (SEW-Eurodrive, Hayward, CA.) used to measure the swimming performance of fish during experimentation. Both swimming flumes are equipped with a fish swimming chamber, as well as a honeycomb filter, veins and cross-wings that aid in developing laminar flow.

Data Analyses

The majority of data did not meet assumptions (i.e., normal distribution, equal variance) to model using parametric statistics, and non-parametric alternatives to ANOVA (ANOVA on rank transformed data) were performed (Iman and Conover 1976). A Two-Way ANOVA on ranked data was used to test for differences in weights (g) of fish and water temperature (°C) across treatments and months of experimentation. Because there were significant differences in fish size and temperature across months of testing, Two-Way ANOVA on Ranks within each month was used to test for differences in treatment condition × response variable (% 168 h survival, % external tissue damage, and blood plasma constituents [Hct, cortisol, glucose, and lactate]). As a result of differences in fish size across months of testing, fish swimming data is reported and was analyzed as seconds swam at a velocity of 80 cm/s per mm of fish (sec/mm). Statistical analyses was be performed using Sigmastat 3.0 (Jandel Scientific, San Rafael, California) software package. Differences were be considered significant at $P < 0.05$.

Results and Discussion

Fish weights (g) and total lengths (mm) were not different across treatments within each month for all measured variables, but mean fish size (length, weight) increased with month of testing from April (87.1 mm, 5.2 g) to May (92.1 mm, 6.1 g) to June (101.2 mm, 7.9 g). Mean post-treatment two-hour holding treated water temperatures and SSJD water temperatures in April, May, and June, were 15.7 and 14.9°C, 15.8 and 15.8°C, and 20.1 and 19.9°C, respectively.

Physiological Stress Response

Month of testing had a significant effect on all measured Chinook Salmon blood plasma constituent levels, with cortisol and lactate levels increasing with month, when averaged over treatment condition. Within each month and sample period (0, 2, and 168 h) effects of handling and clipping procedures, as well as post-treatment holding environment (treated or pure SSJD water), had no significant effect on cortisol (ng/mL), glucose (mg/dL), lactate (mg/dL) or hematocrit level (Figure 6, 7, 8, and 9, Two Way ANOVA on Ranks, $P < 0.05$). Handling only and handling and clipping procedures did not result in a significant immediate increase in cortisol or glucose levels. Regardless of month tested, salmon cortisol and glucose levels followed the same post-treatment temporal pattern, with levels peaking at 2 h post-treatment and then generally returning to control levels by the 168-h sampling period. However, peak glucose levels at 2 h post treatment were generally not significantly greater then samples acquired at 0 h.

Across all months of testing, handling only and handling and clipping resulted in an increase in lactate levels, and, in general, these levels remained elevated through 2 h and returned to pre-treatment control levels within 168 hours. In general, mean Hct levels tended to decrease, from 0 h to 2 h sampling periods, and then increase to, or above, 0 h levels at 168 h post-treatment, regardless of treatment condition. However, this temporal trend was only significant in June, and was only consistent for handled and clipped and control fish.

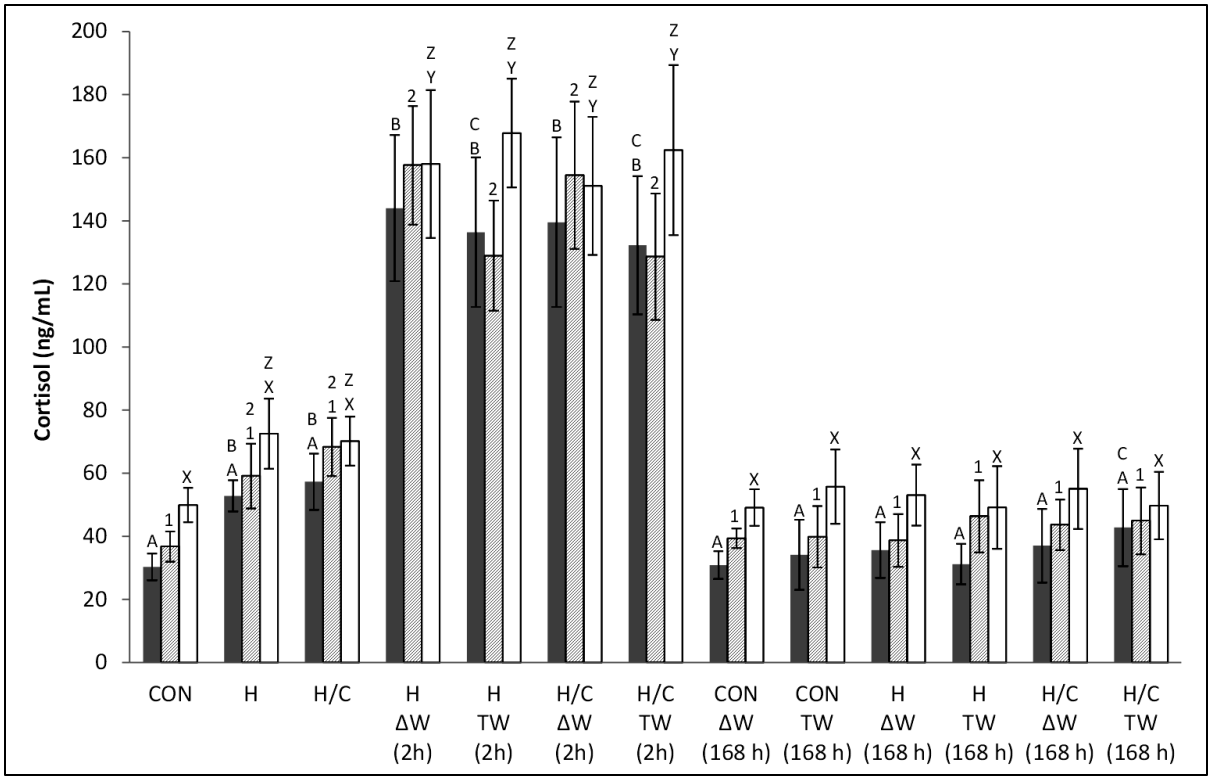

Figure 6.—Mean (± 2 × standard error) blood cortisol levels (ng/mL) of juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) tested in April (grey), May (striped), and June (white) following standard handling (H) or clipping (H/C) operations at California's Central Valley state and federal water project facilities to acquire tissue samples for DNA analyses. Following handling or clipping fish were maintained for two hours in either treated (4 ppt NaCl, 0.2 mL/L PolyAqua) water (TW) or pure Sacramento-San Joaquin Delta water (ΔW) then transferred to 168-h survival tanks where they were exposed to treated water (ozonated and ultraviolet sterilized) ΔW or pure ΔW. Differences in treatment, but within month, are denoted by the following for each month of testing: April (A,B,C), May (1,2), June (X,Y,Z).

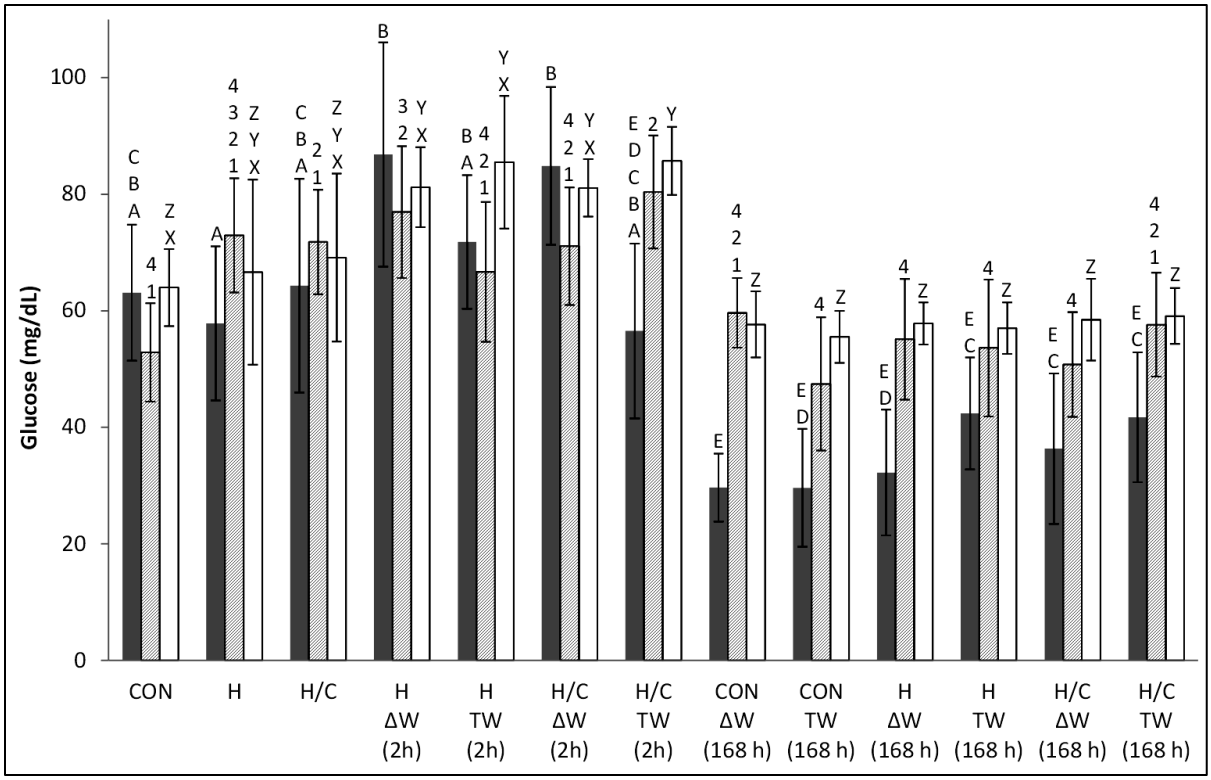

Figure 7.—Mean (± 2 standard error) blood glucose levels (mg/dL) of juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) tested in April (grey), May (striped), and June (white) following standard handling (H) or clipping (H/C) operations at California's Central Valley state and federal water project facilities to acquire tissue samples for DNA analyses. Following handling or clipping fish were maintained for two hours in either treated (4 ppt NaCl, 0.2 mL/L PolyAqua) water (TW) or pure Sacramento-San Joaquin Delta water (ΔW) then transferred to 168-h survival tanks where they were exposed to treated water (ozonated and ultraviolet sterilized) ΔW or pure ΔW. Differences in treatment, but within month, are denoted by the following for each month of testing: April (A, B, C, D, E), May (1, 2, 3, 4), June (X, Y, Z).

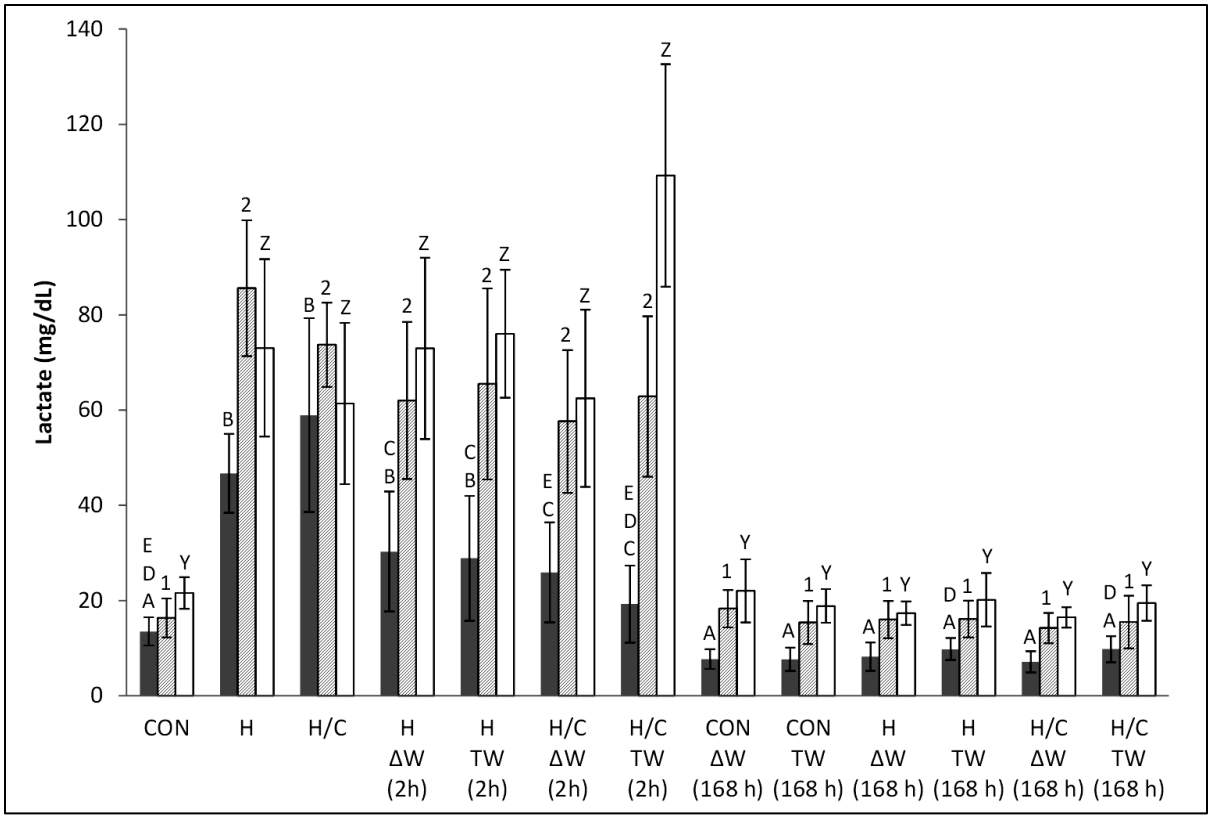

Figure 8.—Mean (± 2 standard error) blood lactate levels (mg/dL) of juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) tested in April (grey), May (striped), and June (white) following standard handling (H) or clipping (H/C) operations at California's Central Valley state and federal water project facilities to acquire tissue samples for DNA analyses. Following handling or clipping fish were maintained for two hours in either treated (4 ppt NaCl, 0.2 mL/L PolyAqua) water (TW) or pure Sacramento-San Joaquin Delta water (ΔW) then transferred to 168-h survival tanks where they were exposed to treated water (ozonated and ultraviolet sterilized) ΔW or pure ΔW. Differences in treatment, but within month, are denoted by the following for each month of testing: April (A, B, C, D, E), May (1, 2), June (Y, Z).

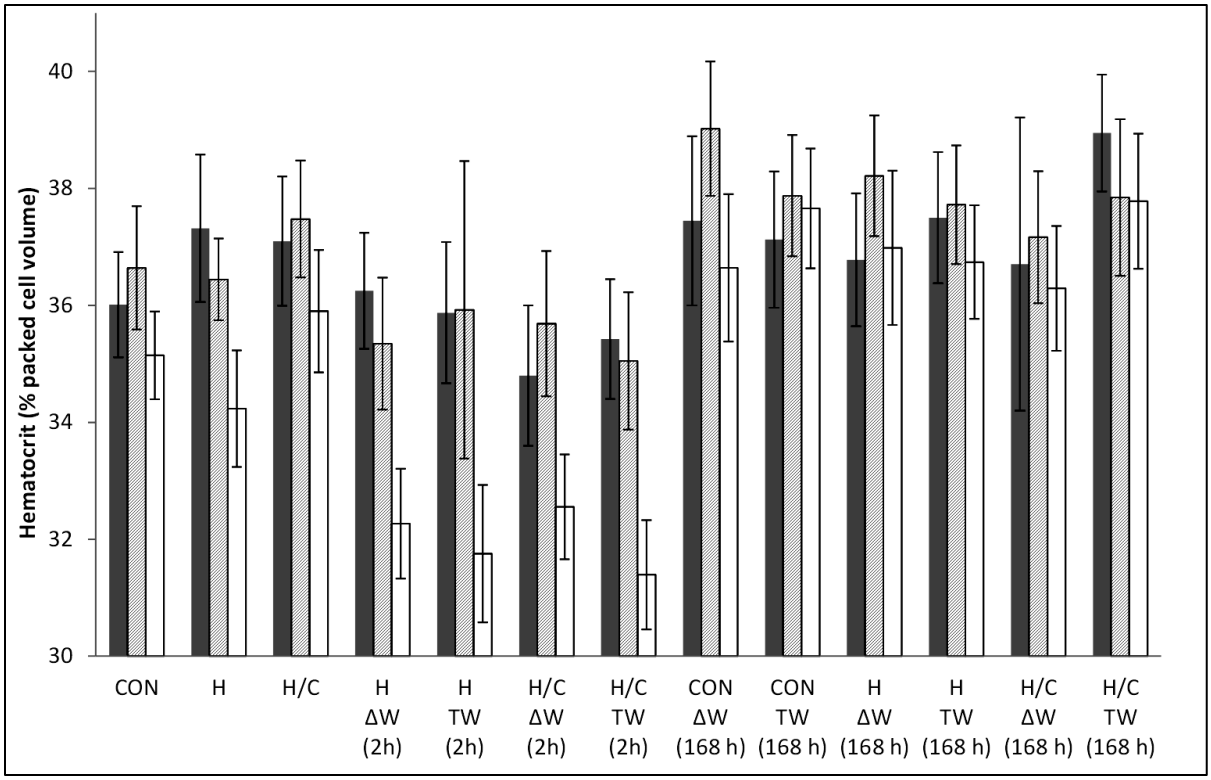

Figure 9.—Mean (± 2 standard error) hematocrit levels (% packed cell volume) of juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) tested in April (grey), May (striped), and June (white) following standard handling (H) or clipping (H/C) operations at California's Central Valley state and federal water project facilities to acquire tissue samples for DNA analyses. Following handling or clipping fish were maintained for two hours in either treated (4 ppt NaCl, 0.2 mL/L PolyAqua) water (TW) or pure Sacramento-San Joaquin Delta water (ΔW) then transferred to 168-h survival tanks where they were exposed to treated water (ozonated and ultraviolet sterilized ΔW) or pure ΔW.

Mean Hct levels, regardless of treatment condition or time following handling and clipping, were within the range generally reported for freshwater fish, suggesting they were healthy and not likely predisposed to pathogenic infection (Wedemeyer et al. 1990; Barton et al. 2002). Pre-stress cortisol (Strange et al. 1978; Strange and Schreck 1980; Barton et al. 1986), glucose and lactate levels (Barton et al. 1986; Barton et al. 2002) of test fish were below or within the range of values typically reported for resting Chinook Salmon and other fishes, suggesting pre-treatment holding environment did not expose fish to inordinate stress. Peaking cortisol and lactate levels shortly (< 4 h) post-stressor, then returning to control levels within 168 h, were similar in magnitude and duration to those reported for Chinook Salmon exposed to similar handling stressors (Barton et al. 1986; Maule et al. 1989; Mesa 1994). Interestingly, handling stressors typically result in increased glucose levels in salmon (Barton et al. 1986; Mesa 1994), which was not observed in the current study. However, mobilization of glucose into the blood following stress in salmonids is gradual and may have peaked after our 2 h sample period (Barton et al. 1986; Barton 2000).

Experimental results indicate there was no difference in physiological stress of salmon exposed to handling only or handling and clipping, suggesting fin-clipping alone provided no significant additional stress, and netting, anaesthetization, and handling fish is probably the most stressful component of the fin-clipping process. This is supported by others who have suggested netting

and handling of fish is the dominant cause of stress during multi-component procedures (Specker and Schreck 1980; Maule et al. 1988). Also, addition of NaCl and conditioner to post-treatment water did not lessen the magnitude of post-treatment cortisol, glucose or lactate levels. Barton and Peter (1982) and Barton and Zitzow (1995) also indicated addition of NaCl did not significantly reduce magnitude of salmonid cortisol levels in response to stress. When exposed to stressors, fish gill permeability generally increases, leading to osmoregulatory imbalances (Mazeaud et al. 1977). The addition of NaCl to water during or following stressors (i.e., netting, handling, etc.) near the internal plasma concentration of fish minimizes the energetic requirements of osmoregulation (Redding and Schreck 1983). Though the physiological benefits of NaCl during handling and clipping is not evident based on the parameters measured in the current study, osmoregulation (i.e., osmolality) of fish in other studies with (Barton and Zitzow 1995) and without (Barton et al. 1986; Barton et al. 2002) the addition of NaCl during or following stressors supports the use of NaCl during all salmon fin-clipping procedures.

External Tissue Damage

When assessed within each month and sampling period (0, 2, or 168 h) clipping did not contribute to an increase in tissue damage compared to handling alone, and the use of treated water did not minimize tissue damage. Percent tissue damage, across all months, was highest when immediately sampled following handling and clipping (Figure 10). However, this relationship was only significant in May with handled only salmon. During April and June testing, tissue damage was lower than control levels, regardless of holding environment, at 2 h post-treatment. When evaluated through the duration of testing (May, June) tissue damage was the same as control levels at 168 h post-treatment. Percentage of external tissue damage, as a result of handling and clipping, varied significantly across months of testing (Figure 10, Two-Way ANOVA on Ranks; $P < 0.05$). This difference was most evident during 0 h and 2 h sampling periods.

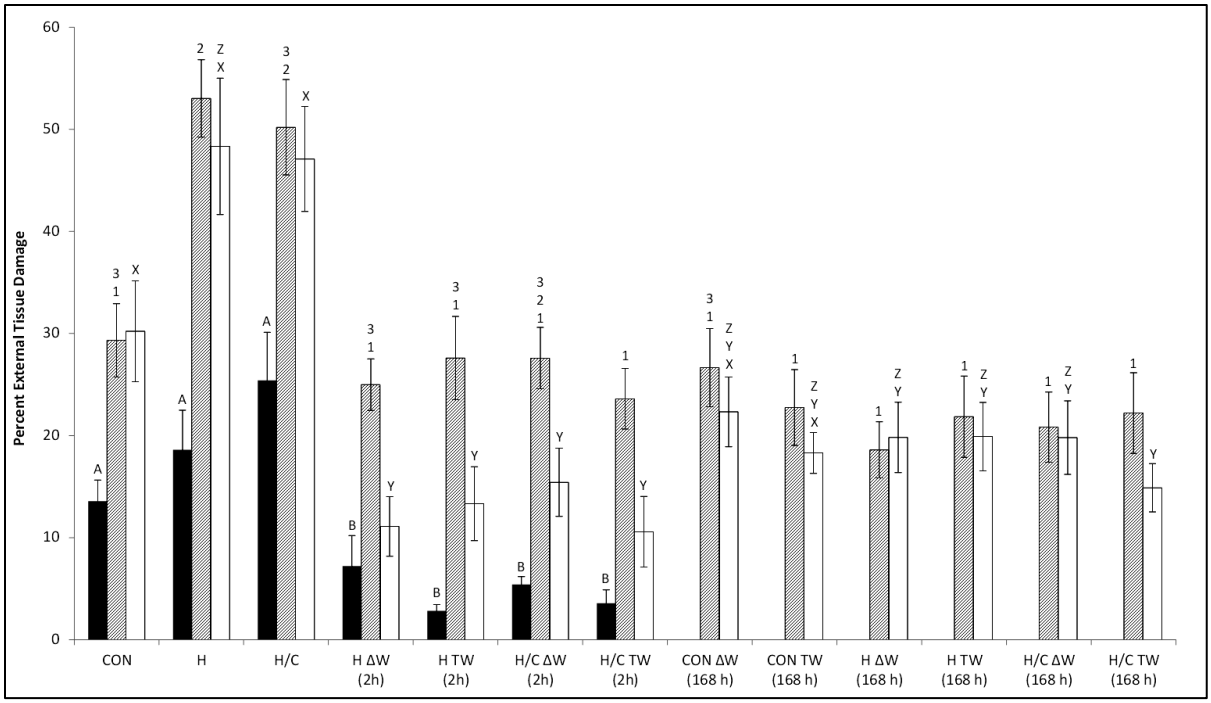

Figure 10.—Mean (± 2 standard error) percent tissue damage, as assessed using flourescein, of juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) tested in April (grey), May (striped), and June (white) following standard handling (H) or clipping (H/C) operations at California's Central Valley state and federal water project facilities to acquire tissue samples for DNA analyses. Following handling or clipping fish were maintained for two hours in either treated (4 ppt NaCl, 0.2 mL/L PolyAqua) water (TW) or pure Sacramento-San Joaquin Delta water (ΔW) then transferred to 168-h survival tanks where they were exposed to treated water (ozonated and ultraviolet sterilized ΔW) or pure ΔW. When averaged across treatment condition, percent external damage was different across each month of testing (Two-Way ANOVA on Ranks; $P < 0.05$). Differences in treatment, but within month, are denoted by the following for each month of testing: April (A, B), May (1, 2, 3), June (X, Y, Z).

External tissue and fin damage, as well as loss of the epidermal mucus layer, resulting from handling and netting, capturing, and handling fish, as observed in the current study is common (Cooke et al. 1998; Cooke and Hogle 2000; Barthel et al. 2003). However, based on a review of the pertinent scientific literature, this is the first study to quantify duration of external tissue damage following netting and handling. During post-treatment examination of test fish, there were no noticeable lacerations or ulcers detected. As a result, the majority of external damage to handled and clipped salmon was assumed to be a result of removal of the epidermal mucus layer. Interestingly, juvenile salmon appeared to be able to regenerate their epidermal mucus layer within 2 h following exposure to fin-clipping procedures. This is important when considering effects of netting, handling, and fin-clipping, because though the epidermal mucus layer is multifunctional, it serves as a protective layer against pathogenic infection (Shepherd 1994; Hellio et al. 2002; Subramanian et al. 2008).

As was observed when quantifying physiological effects of fin-clipping salmon, no additional external tissue damage was evident as a result of fin-clipping procedures compared to handled only fish. Interestingly, salmon maintained in water with or without addition of NaCl and water conditioner, intended to provide an artificial epidermal mucus layer, were equivalent, or below, their pre-treatment (control) tissue damage levels at 2 h post handling only or handling and clipping. Perhaps immediate use of commercially available protective coating following netting

and handling could reduce immediate tissue damage incurred to fish as a result of fin-clipping procedures. However, that was not evaluated in the current study.

Fish Swimming Performance

Chinook Salmon morphometric characteristics and fish swimming performance experimental conditions, including percentage of non-participating fish, as a function of treatment condition are reported in Appendix A, Table A-1. Month and treatment had a significant effect on swimming performance of juvenile Chinook Salmon (Two-Way ANOVA on Ranks, $P < 0.05$). Treatment had no effect on the swimming performance of fish in April or June (Figure 11). In May fish exposed to handling (H) and immediately tested had better swimming performance compared to all other treatments except fish exposed to handling and clipped (H/C) and immediately tested for swimming performance (Two-Way ANOVA on Ranks, $P < 0.05$). Comparisons across months tested within each treatment group are summarized in Appendix A, Table A-2.

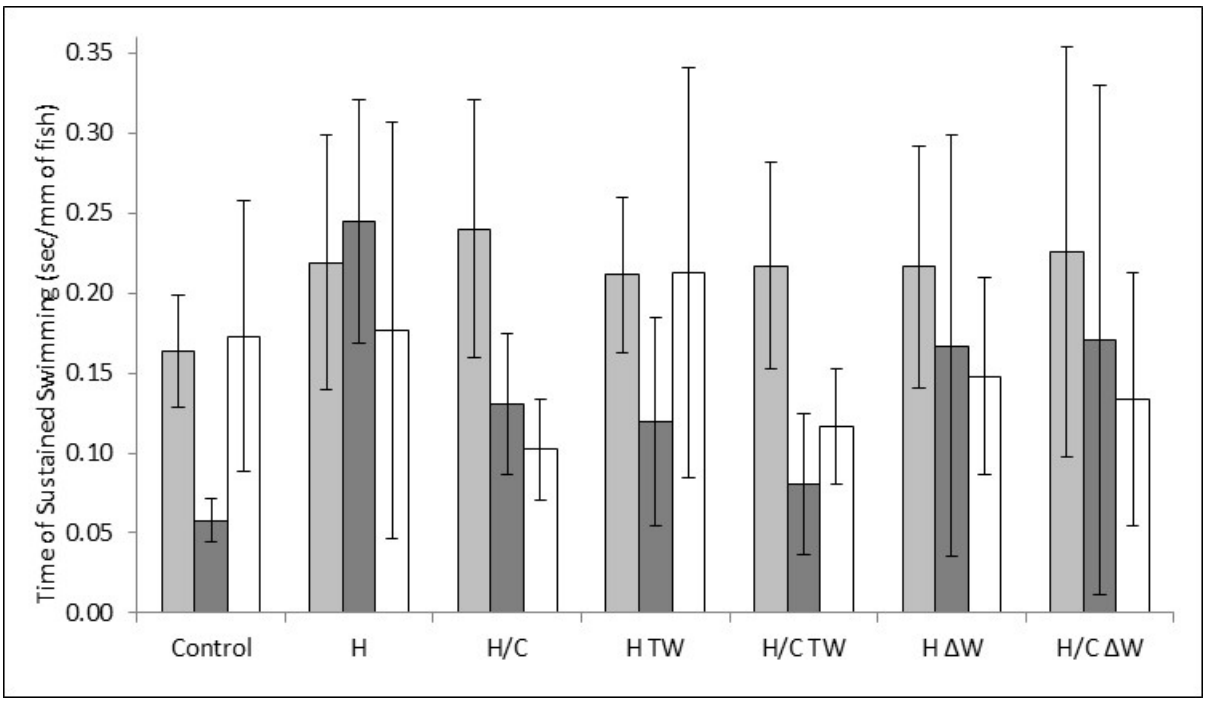

Figure 11.—The swimming performance of Chinook Salmon, reported as seconds sustained at 80 cm/s per mm of fish (mean ± 2 standard error), during experimentation in April (light grey), May (dark grey), and June (white). There were no differences in swimming velocities of fish across treatments in April and June, but swimming performance of salmon in May and exposed to the handled only treatment (H) were greater than all other treatments except handled and clipped treatment (H/C; Two-Way ANOVA on Ranks, $P < 0.05$, $\alpha = 0.05$).

Reports on the effects of handling, associated with fin-clipping of fish, on the swimming performance of fish generally support our results, and indicate typical handling procedures (netting, measuring, etc.), as well as anaesthetization using MS-222, has no adverse effects on fish swimming performance (Ward 2003). Effects of clipping caudal fin lobes on the swimming performance of fish is somewhat incongruous. Radcliffe (1950) reported partial removal of the caudal fin of Goldfish (*Carassius auratus*) had no effect on sustained swimming performance.

However, Horak (1969) and Ward (2003) indicated removal of the majority of a caudal fin lobe of juvenile Rainbow Trout (*O. mykiss*) and Bonytail Chub (*Gila elegans*), respectively, adversely affected swimming ability. Fin-clipping of Chinook Salmon by Fish Diversion Workers at the TFCF resulted in a small area ($\leq 2 \times 4$ mm section) of the caudal fin lobe being removed. It is likely removal of the majority of the fin, as per the methodologies employed by Horak (1969) and Ward (2003), compared to a small portion of the fin, may impair the burst swimming performance of Chinook Salmon.

168-h Survival

Survival of Chinook Salmon, regardless of treatment condition, throughout the 168-h post-treatment assessment period was high (> 94%), and, in general, handling and clipping procedures had no significant effect on survival (Figure 12). However, survival of juvenile Chinook Salmon were influenced by post-treatment holding environment and varied as a function of testing period. This was pronounced in June, at the highest tested water temperatures, when salmon exposed to pure SSJD water exhibited higher mortality compared to sterilized SSJD water. Extensive anthropogenic development in the Central Valley of California has contributed to SSJD water that can have high concentrations of pathogens, as well as other potentially hazardous pollutants, that can be harmful to fish (Lee and Jones-Lee 2004). However, ultraviolet light used to treat SSJD water during experimentation denatures DNA of microorganisms, including bacteria and viruses, resulting in loss of function or mortality (Chang et al. 1985; Summerfelt et al. 2001). Though dependent on pathogen type, elevations in temperature can contribute to reduced disease resistance and increased mortality in salmonids (Holt et al. 1975; Udey et al. 1975). Test temperatures (pre- and post-stressor) in June were likely outside of the preferred range for juvenile Chinook Salmon (Brett 1952; Sauter 1996), and may have even been approaching upper thermal levels (Olson and Foster 1957; Hanson 1991), which likely resulted in thermal stress. Given stress can impair immune performance in salmon (Maule et al. 1989), thermal stress likely contributed to increased mortality when fish were maintained in unsterilized SSJD water.

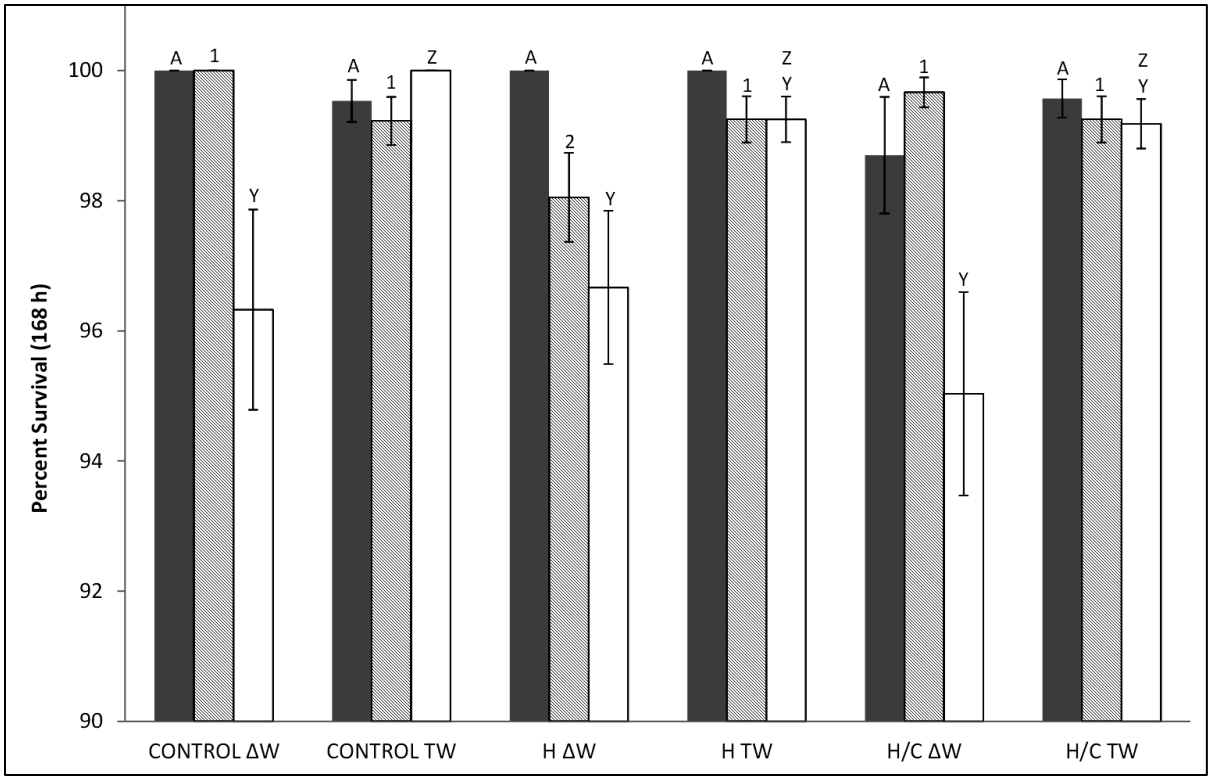

Figure 12.—Mean (± 2 standard error) percent survival (168-h) of control Chinook Salmon (*Oncorhynchus tshawytscha*; CONTROL), or following handling (H) or handling and clipping (H/C) procedures used at California Central Valley water project facilities to acquire tissue samples for DNA analyses. Fish were tested in April (dark grey), May (striped), and June (light grey), and exposed to post-treatment holding in either pure Sacramento-San Joaquin Delta (SSJD) water (ΔW) or ozonated and ultraviolet sterilized SSJD water (TW). Month of testing significantly affected percent survival. Differences in treatment, but within month, are denoted by the following for each month of testing: April (A), May (1, 2), June (X, Y).

Conclusions

Results of the current study suggest physiological stress, and some external tissue damage, as a result of typical fish netting and handling necessary during fin-clipping, are likely unavoidable (Barton et al. 2002). However, when care is taken to ensure fish handling materials and procedures are not extraneous, salmon response is adaptive, and they are able to recover from physiological stress and moderate tissue damage or loss of epidermal mucous layer rapidly (Sharpe et al. 1998). Other research suggests the additional of NaCl benefit fish during and following handling stress and should be used when handling and transporting fish at the TFCF. The adaptive physiological stress response promotes internal homeostasis and immune function (Mommsen et al. 1999), while regeneration of the epidermal mucous layer assists in resistance to harmful pathogens (Shepherd 1994), which, as our results indicate, contributes to high survival. Though experimental results suggest high 168-h survival for fish exposed to all treatment conditions (> 94 %), data suggests holding environment (i.e., water quality) may impact salmon survival at elevated water temperatures.

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Appendix A.—Additional Data Collected on Effects of Handling Only and Handling and Clipping on Burst Swimming Performance of Chinook Salmon (*Oncorhynchus tshawytscha*)

Table A-1.—Morphometric characteristics, sample size, and % of fish for each treatment condition were deemed to have not participated (DNP) when evaluating the effects of handling (H) or handling and clipping (H/C) on burst swimming performance of juvenile Chinook Salmon (*Oncorhynchus tshawytscha*). Following handling and/or clipping, fish were provided two hours to recover in either treated (TW) or raw Sacramento-San Joaquin Delta Water (∆W) before again evaluating burst swimming performance.

	Control	H	H/C	H TW	H/C TW	H ∆W	H/C ∆W
April							
Temperature (°C)	15.3 ± 0.3	15.4 ± 0.5	15.5 ± 0.8	15.6 ± 0.6	15.5 ± 0.4	15.6 ± 0.6	15.7 ± 0.5
Weight (g)	5.4 ± 1.1	5.9 ± 1.0	5.8 ± 0.8	5.6 ± 0.8	5.5 ± 1.0	5.5 ± 0.9	5.6 ± 0.9
Total Length (mm)	83.6 ± 6.3	86.2 ± 5.6	86.3 ± 3.8	86.4 ± 3.7	85.6 ± 4.8	85.4 ± 5.2	84.7 ± 4.7
Sample Size (n)	37	17	22	20	20	19	19
% DNP	21.6%	5.9%	4.5%	10.0%	20.0%	0.0%	10.5%
May							
Temperature (°C)	15.6 ± 0.3	15.6 ± 0.3	15.6 ± 0.3	15.7 ± 0.3	15.7 ± 0.3	15.7 ± 0.2	15.7 ± 0.2
Weight (g)	6.1 ± 1.2	6.4 ± 1.1	6.6 ± 0.8	6.5 ± 0.9	6.7 ± 1.2	6.7 ± 1.1	6.3 ± 1.4
Total Length (mm)	88.8 ± 6.2	89.8 ± 5.6	90.8 ± 3.9	90.0 ± 4.4	91.1 ± 6.0	92.0 ± 5.0	88.7 ± 6.2
Sample Size (n)	36	24	24	24	24	24	24
% DNP	44.4%	4.2%	20.8%	33.3%	33.3%	29.2%	16.7%
June							
Temperature (°C)	20.5 ± 1.0	20.2 ± 0.4	20.3 ± 0.4	20.2 ± 0.5	20.2 ± 0.5	20.2 ± 0.5	20.2 ± 0.5
Weight (g)	8.3 ± 2.1	8.3 ± 1.4	8.7 ± 1.7	9.4 ± 1.9	8.4 ± 2.4	8.5 ± 1.8	7.5 ± 1.6
Total Length (mm)	97.7 ± 8.5	98.8 ± 5.4	99.4 ± 6.3	102.4 ± 6.6	97.6 ± 8.7	98.5 ± 7.1	95.0 ± 6.8
Sample Size (n)	42	24	24	24	24	24	24
% DNP	35.7%	16.7%	29.2%	16.7%	29.2%	25.0%	12.5%

Table A-2.—Results (P-Value) of statistical comparisons (Two-Way ANOVA on Ranks) quantifying effects of control, handled and clipped (H/C), and handled only (H) on juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) burst swimming performance. Following handling and/or clipping, fish were provided two hours to recover in either treated (TW) or raw Sacramento-San Joaquin Delta water (∆W) before again evaluating burst swimming performance.

Comparison	Control	H	H/C	H TW	H/C TW	H ∆W	H/C ∆W
April vs. May	**<0.001**	**0.022**	**0.004**	**<0.001**	**<0.001**	**0.038**	**0.005**
April vs. June	0.346	0.893	0.048	0.221	**0.028**	0.176	**0.006**
June vs. May	**<0.001**	0.12	0.655	0.056	0.092	0.772	0.996