

1 Evaluation of Electroneuroleptosis as a Tool for Collection of Biological Data from Adult Steelhead

2 *Oncorhynchus mykiss*

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36 **Abstract**

37 We evaluated electronarcosis (EN) as an alternative to traditional techniques for
38 immobilizing adult steelhead during trapping operations designed to collect broodstock for
39 hatchery operations and gathering data for a variety of management and research activities. We
40 compared the rates of fish injury, adult pre-spawn mortality, embryo survival, and spawner
41 handling efficiency of the handling between EN and standard operating procedures with and
42 without chemical anesthetics. Similar rates of injuries occurred in adult steelhead when EN and
43 tricaine methanesulfonate (MS-222) were used to immobilize fish. Similar rates of mortality
44 were observed for developing embryos from parents treated with EN or MS-222. In
45 comparisons between EN and a V-trough without anesthetic, the use of an EN chamber allowed
46 for scale sampling procedures to be completed faster than V-troughs. Fish sex and handling
47 treatment contributed significantly to the models describing survival distribution of steelhead
48 handled using EN or VT without anesthetic. A lower mortality rate in females treated with EN
49 than the V-trough treatment was observed. These data support the further use and evaluation of
50 EN on steelhead when collecting data, biological samples, or broodstock of salmonids in field or
51 hatchery settings.

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Introduction

56 Large numbers of adult salmonids are captured and handled annually throughout the
57 Pacific Northwest portions of North America to collect broodstock for hatchery programs or to
58 collect data that contribute to understanding population status. Handling adult salmonids safely
59 during these procedures can be difficult for these purposes without some form of anesthesia or
60 platform to restrain fish movements. Currently, tricaine methanesulfonate (MS-222) is the only
61 chemical anesthetic approved by the United States Food and Drug Administration (USFDA) for
62 use on fish (Trushenski et al. 2013). However, the label requires a 21-day withdrawal period and
63 complicates practices of immediate release after sampling. Another anesthetic, Aqui-S 20E
64 (AquaTactics Fish Health, Kirkland, WA, USA), allows for immediate release following field
65 based applications but its use in the USA is currently restricted to participants of an
66 Investigational New Animal Drug permit (USFDA INAD 11-741) requiring detailed reporting of
67 its use and effectiveness. Carbon-dioxide (CO₂) can be used as a fish anesthetic and is
68 considered a Low Regulatory Priority compound by the USFDA (USFDA 2011) and thus allows
69 for immediate release but it can be cumbersome to apply in remote field settings and
70 hyperactivity of fish upon exposure to CO₂ charged water (Bell 1987) has injury potential to both
71 fish and workers. Additionally, wide ranging physiological impact is reported in instances of
72 hypercapnia in fishes (Tufts and Perry 1998) that contributes to a slow recovery from exposure
73 to CO₂ charged water (Wagner et al. 2002; Pirhonen and Schreck 2003). Procedures used in
74 various locations also include sampling platforms that restrain fish movements without the use of
75 chemical anesthesia. The V-trough used by Washington Department of Fish and Wildlife
76 (WDFW; Figure 1) is an example of a platform that limits fish movement to promote efficient
77 sampling and frequently used without anesthesia. Fish routinely attempt to escape the V-trough

78 forcing the worker to expend additional effort to restrain fish while sampling. This practice can
79 add risks of injury to both the fish and the worker while increasing the amount of time necessary
80 to collect samples and data.

81 Electricity is an alternative for immobilizing fish for handling and sampling practices.
82 Electroanesthesia (EA) is conducted routinely with modified electrofishing equipment using
83 direct current (DC) or pulsed DC at voltages ranging 100-300 V (Tipping and Gilhuly 1996; Cho
84 et al. 2002; Vandergoot et al. 2011; Faust et al. 2017). Some studies suggest that EA represents a
85 useful alternative to chemically induced anesthesia with adult response and egg survival within
86 acceptable performance levels for a production hatchery and research settings (Tesch et al. 1999;
87 Jennings and Looney 1998; Trushenski et al. 2012). However, numerous studies that include
88 data for a variety of fish species have described the deleterious effects of EA that include
89 intramuscular hemorrhage, vertebral injuries, reduced gamete viability, reduced juvenile growth
90 rates, delayed hatching, and increased mortality (Sharber and Carothers 1988; Dwyer et al. 1993;
91 Hollender and Carline 1994; Thompson et al. 1997; Ainslie et al. 1998; Redman et al. 1998;
92 Keefe et al. 2000). The majority of these EA studies utilized technology that relied upon
93 voltages > 100-120 V. The use of lower DC voltages (≤ 60 V DC) to conduct fish research and
94 fish culture activities has been revisited (Hudson et al. 2011; Vandergroot et al. 2011; Faust et al.
95 2017). This lower voltage approach is distinguished from EA by the responses of the fish to the
96 electric field and has been termed, electronarcosis (EN). While EA results in a persistent
97 quiescent period lasting up to several minutes, EN induces immobilization and muscle relaxation
98 of the fish only while it is held in the electric field (Vibert 1963). As such, EN allows for sorting
99 and collecting potential broodstock with a near immediate recovery upon release.

100 Many locations where EN would be considered contain fish populations protected by the
101 Endangered Species Act where new protocols for handling these sensitive species requires
102 careful screening for negative impacts. Many authors (Cho et al. 2002; Schill and Elle 2000;
103 Tipping and Gilhuly 1996; Sharber and Carothers 1988) have recommended studies on the long-
104 term effects of EA and EN on the target organism prompting investigations on the application to
105 salmonid species. Keep et al. (2015) found no difference in migratory behavior of adult Chinook
106 Salmon *Oncorhynchus tshawytscha* following EN treatment relative to carbon dioxide
107 treatments. Hudson et al. (2014) observed that survival of embryos produced from adult Coho
108 Salmon *O. kisutch* treated with EN was similar to those from MS-222 treated adults. In these
109 studies, EN was considered preferable to carbon dioxide or MS-222 due to faster induction and
110 recovery times. The objectives of this study were to examine the responses of pre-spawning
111 adult summer-run steelhead *O. mykiss* following EN treatments compare these observations to
112 established practices used to handle these fish (MS-222 or V-trough), compare injuries to
113 spawning adult fish exposed to EN versus MS-222, and the resultant embryo survival, and
114 characterize survival distributions of adult summer-run steelhead following the use of EN or V-
115 trough (with no anesthetic).

116 **Methods**

117 *Study location and broodstock collection.* – Summer-run steelhead broodstock were
118 trapped in the fall (Sep-Nov) following entry to a fish ladder at the Lyons Ferry Hatchery (LFH)
119 outfall into the Snake River in 2010 and 2011. Fish were sorted from the adult trap by hatchery
120 staff using a series of pipes with hydraulic gate valves that direct fish back to the Snake River
121 when not needed or into adult holding raceways when they are selected for broodstock. LFH
122 adult holding raceways are 3.1 x 24.4 x 1.8 m with a water flow of 3,785 liters/minute. The

123 water supply at LFH is specific pathogen free well water with a constant temperature of ~11°C.
124 Water conductivity at LFH was recorded between 220-240 µS/m.

125 *Electronarcosis Chambers.* – WDFW personnel constructed two different EN chambers
126 (Figure 1) for these studies. Both chambers received electrical current from a Protek 3006B DC
127 Power Supply (0-60 V, 1.5A; Protek Devices, Tempe, AZ). One of the chambers (CH1) was
128 constructed using a 90 cm section of 21.3 cm diameter PVC pipe cut open along the top and
129 capped on each end. Round, 21.3 cm diameter, thin-plate aluminum electrodes were inserted
130 into the cap ends of the pipe, which when fitted held the electrodes in place. A small portion of
131 the cap on each end was cut away exposing the aluminum plate, where leads from the EN power
132 supply were attached. A second chamber (CH2) was constructed following the design described
133 by Hudson et al. (2011), using a 130 l (95 cm x 38 cm x 36 cm) Igloo™ cooler. A hose receiver
134 was fitted to allow an input of freshwater, and a standpipe installed to maintain constant water
135 depth.

136 A preliminary exercise to test the efficacy of the EN equipment was performed using
137 CH1 on hatchery origin summer-run steelhead captured from the Touchet River adult trap in
138 Dayton, WA. Several fish (55-70 cm) were captured at the trap and introduced individually to
139 CH1 via dip net and their behavioral responses to settings ranging from 30-60 V DC observed.
140 Water temperature in the Touchet River during this initial testing was ~2 °C and conductivity
141 ranged from 45-50 µS/m. All fish reached a state of EN that permitted sampling the animal at or
142 above 50 V DC output. The best results obtained when settings were at 60 V DC. Fish behavior
143 in the chamber included a loss of equilibrium with the muscles remaining relaxed with a regular
144 opercular rate that was similar to that reported by Hudson et al. (2011). Upon removal from the
145 electrical field, fish resumed their normal orientation and were capable of swimming nearly

146 immediately (< 3 s). Based on these results, power supply settings for subsequent tests at LFH
147 were established at 60 V output and not exceeding 0.15 amps representing estimates for output at
148 0.66 V/cm in CH1 and 0.63 V/cm in CH2.

149 *Electronarcosis versus MS-222.* – Summer-run steelhead broodstock (44 - 87 cm) were
150 collected from the LFH fish ladder/adult trap from September to November, 2010. All fish
151 retained for broodstock were sorted on 17 November 2010. A divider panel was placed in the
152 middle of the adult holding raceway during sorting, with females retained on the upstream side,
153 and males on the downstream side. On 28 December 2010, all retained females were sorted
154 again, with any ovulating females removed. On that same date, 40 females and 60 males were
155 transferred to an adjacent adult holding raceway, making up the group of fish treated with EN
156 during spawning operations. Prior to the first spawn date, two females and six males had died in
157 the raceway leaving 38 females and 54 males for treatment with EN. Weekly inspections of all
158 fish to determine those ready to spawn in both raceways commenced on 11 January 2010.

159 During the first inspection, groups of 3-4 fish were placed in the EN unit (CH2) to
160 simulate what might be experienced during hatchery sorting or at a fish trap for broodstock
161 collection or tagging. All fish during this first time in the EN unit were exposed to the electric
162 field for at least one minute. After one minute, fish were removed individually and checked for
163 readiness to ovulate or to shed milt. If fish were determined ready for gamete collection they
164 were euthanized with a sharp blow to the head and placed on a spawning rack until gametes were
165 collected. If the fish were not ready for gamete collection they were injected with a passive
166 integrated transponder (PIT) tags into the dorsal sinus and returned to the adult holding raceway.
167 PIT tags were only applied to the EN group to identify individuals so that the cumulative amount
168 of time exposed to the electric field over the course of the seven-week spawning period be

169 determined. Times for this initial EN exposure varied from 60 to 150 s (average time 85 s, SD =
170 23 s). In subsequent weeks, groups of 3-4 fish were transferred into the EN unit but only
171 remained there until they could be inspected for spawning readiness and PIT tag number
172 recorded (average time 41 s, SD = 21 s). Fish selected for gamete collection were processed as
173 described previously and fish not ready were returned to the holding raceway.

174 A second group maintained during this study was represented by broodstock held in the
175 adjacent raceway and inspected for spawning readiness using MS-222 anesthesia. This group of
176 males and females were crowded in the raceway and groups of 5-10 fish were netted and placed
177 into a ~380 l galvanized metal trough filled with ~230 l water containing 19 g of dissolved MS-
178 222 (Tricaine-S, Western Chemical, Ferndale WA). At this dosage, fish reached a state of lost
179 equilibrium within 1-2 minutes before initiating inspections to determine readiness for gamete
180 collection or returned to the adult holding raceway; the entire time to work through 5-10 fish
181 using MS-222 was 5-10 minutes. Eggs from all ovulating females from both EN and MS-222
182 groups were stripped directly into 4-l buckets. Milt from males of both groups were collected in
183 59 ml Whirlpak® bags that were sealed after the addition of oxygen and placed into a cooler.
184 These procedures for both groups were repeated weekly for seven consecutive weeks thus
185 animals may have been exposed multiple times over the course of the study. At each handling,
186 we identified individual fish treated with EN by recording PIT tag number on each spawning
187 date and recorded the amount of time in the EN chamber to calculate cumulative exposure time
188 (seconds) to the electric field for each animal over the course of the study. Similar data were not
189 recorded for fish treated with MS-222 females as PIT tags were only applied to fish treated with
190 EN.

191 Spawning crosses were restricted to combining gametes from individual male and female
192 fish within each treatment group (EN x EN, MS-222 x MS-222). From spawn weeks 1-7, all EN
193 treated fish that were spawned were filleted, the carcasses photographed, and the injuries
194 recorded as described by Zydlewski et al. (2008). For the MS-222 treated group, ~20 fish each
195 week were selected from spawn weeks 2-5 to be filleted and photographed. No spawning
196 occurred after week five in the MS-222 group, as production levels for the hatchery program had
197 been reached.

198 Fertilization procedures for both treatment groups were as follows. After eggs and milt
199 were combined, ~100 mL of water was added to each bucket to activate sperm and enable
200 fertilization. After 1-2 minutes, the buckets were partially filled with an iodophor solution (100
201 ppm; Ovadine, Western Chemical, Ferndale, WA) and the fertilized eggs then poured through a
202 metal strainer to remove the iodophor solution, excess ovarian fluid, and coagulated blood. Eggs
203 were returned to the bucket, new 100 ppm iodophor solution was added, and the eggs were
204 allowed to harden for one hour. After water hardening, the eggs were then transferred into
205 incubators constructed from two nested 4-gallon square buckets. Water was introduced to the
206 buckets from the bottom of the outside bucket and flowed upward through second bucket
207 containing the eggs through a screen to provide even flow across all eggs. After 14-16 days
208 (161-184 temperature units), the incubating eggs were removed from the buckets and shocked by
209 pouring eggs from the incubation bucket into a second bucket to coagulate nonviable eggs. The
210 following day dead eggs were removed and counted. Weights (0.1 g) of a sample of 300 live
211 eggs and all the remaining live eggs in each bucket were recorded to calculate the number of
212 viable eggs remaining for each spawning pair. Total fecundity for each female was determined
213 as the sum of dead eggs plus live eggs remaining and the percent survival for each spawning pair

214 calculated. After this shocking date, inventoried eggs were pooled into aggregate EN and MS-
215 222 treated groups with no replicates.

216 A Fisher's exact test was used to determine differences in the frequencies of fish with and
217 without observed injuries between EN and MS-222 treated fish. Differences in fecundity and
218 egg survival between EN and MS-222 treated fish were determined using t-tests. Egg survival
219 during incubation is highly variable in LFH summer-run steelhead and frequently deviates from
220 normal distributions. As a result, percent survival for eggs was rank-transformed before the t-
221 tests were performed (Zar 1996). The cumulative impact of EN was determined using rank
222 correlation tests to determine any relationship between egg mortality and : 1) the number of
223 times (1-7) the female fish had been exposed to the electric field, and 2) the cumulative amount
224 of time (s) in the EN chamber over the entire seven week spawning season.

225 *Electronarcosis versus V-Trough sampling.* – WDFW personnel trapped 549 summer-run
226 steelhead broodstock (50-90 cm) at LFH during early October, 2011 for this test. All fish were
227 held in an adult holding raceway and were crowded and sorted on 12 October. Four hundred
228 summer-run steelhead were assigned equally to two groups, the groups consisting of sampling
229 using EN (CH1) or the V-Trough (VT). The VT used by WDFW allows a fish to lie between
230 two panels (each panel angled at $\sim 45^\circ$ off the base of the platform) with the head covered as data
231 and samples are collected without the use of an anesthetic. Individual fish were captured with a
232 dip net, removed from the net and inserted into the VT or netted and held in the net while being
233 immersed into CH1. The transfer of fish alternated between VT or the CH1 as they were
234 collected. Fork length and fish sex were recorded for all fish. A scale sampling procedure was
235 added for 75 fish from each group. These actions mimic handling procedures that would occur

236 at an adult trap for sampling or broodstock collection. The remaining crowded fish in the
237 holding raceway (N=149) were left unhandled and served as a control group (CT).

238 For both EN and VT methods, the time required to collect data and samples (seconds)
239 was recorded for a subset of animals collected at random from the entire population of both
240 treatments. The amount of time to net and insert individual fish into either the EN unit or the
241 VT, determine fish sex, collect fork length data was recorded for 25 EN fish and 38 VT fish.
242 When scale sampling was added to the process, times for 25 fish from both EN and VT groups
243 were recorded. One staff member was assigned to collect data and samples using EN, another
244 staff member collected the same data using the VT, and a third staff member recorded the
245 biological data and sampling times for both groups. Following data collection and sampling, the
246 upper or lower portion of the caudal fins were clipped for later identification of EN and VT
247 treatment groups and returned to the holding raceway. The CT fish that were not
248 handled/sampled received no mark but were subjected to the same raceway crowding as test fish
249 and maintained in the same adult holding raceway. All mortalities were removed daily from the
250 raceway by hatchery staff with data regarding fin clips recorded until the end of the evaluation
251 on January 17, 2012, for a total of 97 d. The number of fish from each group were confirmed at
252 the end of the study through a tally and examination of records of all mortalities and live fish
253 remaining after 97 d. On 16 December 2011, 32 fish were removed (10 EN, 10 VT, 12 CT) and
254 killed for a basic necropsy inspection and these animals were censored from survival distribution
255 analyses described below.

256 Differences in the amount of time to collect data and samples for EN and VT were
257 determined using t-tests. The threshold for significant differences was $P < 0.05$. Patterns of
258 survival over time for the three groups were modeled using parametric and non-parametric

259 statistical procedures that characterize time-to-event data (Lawless 1982; Sandford et. al. 2012;
260 Tableman and Kim 2004). In this study the event was the death of a fish of a particular treatment
261 (EN, VT, and CT) and time (days). We modeled survival distributions using two different
262 procedures. First, we used the non-parametric Kaplan-Meier (KM) method (Lawless 1982) to
263 visualize and make straightforward comparisons of survival for each treatment in a data set
264 containing censored observations. We followed this analysis with parametric modeling
265 approaches using commonly applied distributions to describe survival data: logistic, log-logistic,
266 lognormal, Gaussian, Weibull, and exponential (Tableman and Kim 2004). The parametric
267 approach included evaluation of the factors of fish sex, treatment, and their interaction in
268 determining the best model fit for the survival distribution. For each distribution we fit five
269 models: constant (no explanatory variables), treatment alone, sex alone, both treatment and sex,
270 and both including an interaction term. We calculated a measure of the explanatory information
271 for each model, Akaike's Information Criterion (AIC; Burnham and Anderson 2002) as well as
272 the difference between each model's AIC and the minimum of the five, ΔAIC . We chose the
273 model with the minimum AIC as the model that best described the observed data provided ΔAIC
274 > 2 for the other models. In the instance of $\Delta AIC < 2.0$ we omitted models that added a single
275 term to the best model, as the term was not adding information, rather it was just "benefitting"
276 from being added to the best model (i.e., "pretender variables"; Arnold 2010). For each
277 distribution, we selected the best model and then selected the minimum AIC from this group as
278 the best model from all distributions. Analyses of survival distributions were conducted using R,
279 specifically with the survival package (R Core Team 2017, Therneau 2015).

280

Results

281 *Electronarcosis versus MS-222.* – Injuries consistent with spinal and intramuscular
282 hemorrhage occurred at low frequency and at similar rates in both the EN and the MS-222
283 treated group of adult summer-run steelhead ($P > 0.99$; Table 1). Mean fecundity did not differ
284 significantly between treatment groups ($P = 0.43$; Table 2). Mortality of 100% of the
285 developing embryos was observed between fertilization and the eyed stage in 13.6% of spawned
286 females collected in the MS-222 treated group and 3.1% of spawned females in the EN treated
287 group. Median rates of mortality between fertilization and the eyed stage in EN and MS-222
288 treated fish were 10.5 and 10.0%, respectively. No significant difference in the rank-transformed
289 percent mortality from fertilization to eyed-egg stage between the EN and MS-222 treated
290 groups were observed ($P = 0.11$). Results of the rank correlation test indicate weak negative
291 relationships for both egg mortality versus the number of exposures ($R = -0.25$, $N=31$) and
292 between egg mortality versus cumulative exposure time ($R = -0.12$, $N=31$). Losses from
293 individual spawns were not followed from the eyed stage through hatching as eggs were
294 combined following initial inventory, but mortality between the eyed egg stage and the hatched
295 alevin stage from MS-222 broodstock was 2.7% and in EN treated, 3.1%.

296 *Electronarcosis versus V-Trough sampling.* – A similar amount of time elapsed for
297 sampling fish using EN and the V-trough when no scales were collected, ~25-26 s. However,
298 when scales were collected, the average time required to net, sample, and release fish in the EN
299 group, 40 ± 1 s (Mean \pm SE), was significantly ($P < 0.01$) less than fish processed in the V-
300 trough, 51 ± 2 s.

301 Following return into the raceways, no mortality was observed for any cohort for nearly
302 40 d (Figure 2). At ~40 d, VT females began to die, followed two weeks later by females from
303 other treatment groups. The rate of mortality increased in VT and EN treated males ~2 weeks

304 after it was observed in females and followed finally by an increase in the mortality rate in CT
305 males. At the termination of the study, 97 days after treatment, the surviving proportions among
306 groups of females were 40, 52, and 54% for VT, EN and CT , respectively. Surviving
307 proportions among groups of males were 82, 84 and 90% for EN, VT, and CT , respectively.
308 Parametric survival distribution analyses revealed that regardless of distribution employed in the
309 survival estimation modeling, except for the exponential model, the model of treatment and sex
310 (and no interaction) was best supported by the data (Table 3). We rejected the exponential
311 distribution since the exponential distribution is a special case of the Weibull distribution with
312 scale parameter = 1.0. The estimated scale parameter from our Weibull fit was 0.16. Four of
313 five remaining interaction-term models had ΔAIC near 2.0 (range 1.4-1.8; exception was
314 Weibull at 3.12) but were omitted from further consideration as the edition of the interaction
315 term fit the ‘pretender’ variable description (Arnold 2010). Comparison by AIC of the all the
316 distributions indicated the Gaussian model of fish sex and treatment was best (Table 4). The
317 estimated curves for VT treated fish were very similar to the unhandled controls for both males
318 and females (Figure 2). Mortality in males was very low relative to females and only began
319 dying at an increased rate between days 70 and 80.

320 **Discussion**

321 The EN units developed for these studies were successful at handling and sampling adult
322 summer-run steelhead relative to MS-222 or V-board treatments. As the number of tasks
323 surrounding sampling and data collection increased, the benefits of EN relative to the use of no
324 anesthetic became more apparent. The added time required to sample scales, identify fish sex,
325 and collect length data for the V-board with no anesthetic group are likely the result of a single
326 worker required to better secure the fish while sampling. Handling fish without any anesthesia

327 also had a negative impact on female steelhead vitality relative to the use of EN. Our data
328 illustrate that female steelhead handled using EN had higher survival than females handled using
329 VT without anesthetic. Mortality in females was high relative to males in all groups in this
330 study. Factors contributing to this higher mortality include female fish with diminished vitality
331 as eggs may have matured to a post-ovulatory state combined with the absence of any
332 prophylactic treatments with formalin to control fungus. Immobilization in the absence of
333 anesthesia likely promotes more assertive practices to restrain fish movement leading to a greater
334 rate of surface injuries and loss of surface mucus that promotes fungal infection (Green and
335 Haukenes 2015). Our choice in this study was to avoid confounding variables that would mask
336 the impact of handling treatment such as prophylactic treatments with formalin that would not be
337 available in the context of an immediate release to the environment. We acknowledge that the
338 maintenance of steelhead in concrete raceways without formalin treatments likely contributed to
339 the overall mortality reported here. However, our interpretation of the data, females treated with
340 VT in the absence of chemical anesthesia were compromised and had lower long-term vitality
341 relative to those treated with EN. Complete losses of entire families of progeny between
342 fertilization and inventory (161-184 temperature units) were more frequent among MS-222
343 treated parents than in EN treated parents. Questions surrounding anesthetic use during
344 spawning operations and gamete quality have prompted study on this topic for decades (Billard
345 1980; Redman et al. 1998) but a targeted evaluation of MS-222 impact on egg and sperm quality
346 at relevant concentrations revealed no direct impact (Holcomb et al. 2004). We acknowledge
347 that confounding factors can contribute to losses of eggs incubation trays but our observation
348 should illustrate, at minimum, that there is no evidence of a relationship between exposure time
349 to the electric field and increased embryo mortality and suggest that EN may be a suitable

350 alternative to MS-222 in handling pre-spawning steelhead. Finally, the rates of injury on
351 broodstock were low during spawning operations and comparable to those using MS-222. Our
352 data are consistent with experiments performed on Coho Salmon using similar equipment and
353 also illustrated no effect on EN on progeny of treated parents (Hudson et al. 2014). While not a
354 new technology (Kynard and Lonsdale 1975) reports describing the impacts of EN on fish and
355 their progeny is sparse (Vandergroot 2011; Hudson et al 2014) relative to EA induced by V DC
356 used at higher voltage gradients (Tipping and Gilhuly 1996; Barton and Dwyer 1997; Ainslie et
357 al. 1998; Zydlewski et al. 2008). In aggregate our data on adult steelhead provide further support
358 the continued use of EN in situations where the withdrawal times of chemical anesthetics cannot
359 be controlled.

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Acknowledgments

362 The findings and conclusions in this manuscript are those of the authors and do not
363 necessarily represent the views of the WDFW and the use of commercially available equipment
364 does not denote an endorsement by the authors or the WDFW. We thank McLain Johnson and
365 Tracy Peterson for reviews of earlier versions of this manuscript. The authors also gratefully
366 acknowledge the Lyons Ferry Hatchery staff, especially Doug Maxey, Jon Lovrak, and Steve
367 Jones, and WDFW Snake River Lab staff member Jerry Dedloff for his electronarcosis chamber
368 construction, fish handling, and data collection. Lastly, we are grateful to the Lower Snake
369 River Compensation Plan - USFWS, who provided funding for all this work.

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471 electroshock for euthanizing and immobilizing adult spring chinook salmon in a hatchery.
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473

474

475 Table 1. Incidence of hemorrhaging near the spine and non-spinal for male and female summer-
 476 run steelhead spawned at LFH in 2010. No significant differences ($P > 0.99$) in the frequency of
 477 injuries was observed between chemical anesthesia (MS-222) and electronarcosis (EN).

	MS-222		EN	
	Males	Females	Males	Females
Number examined	40	41	35	32
Number of injuries				
Non-Spinal	4	0	2	0
Spinal	0	1	1	1
Total	4	1	3	1
Percent	6.2%		6.0%	

478

479

480 Table 2. Mean fecundity (eggs/female) for female summer-run steelhead on each spawning date.
 481 No overall difference in fecundity was observed between treatments ($P = 0.43$).

Spawn Date	<u>Electronarcosis</u>		<u>MS-222</u>	
	Number of Females	Fecundity	Number of Females	Fecundity
11 Jan	7	5897	27	5789
18 Jan	7	5241	18	5853
25 Jan	5	5498	20	6321
1 Feb	1	7610	15	5965
8 Feb	6	6471	8	5308
16 Feb	5	5189		
23 Feb	1	5779		
Mean (\pm SD)		5737 \pm 992		5909 \pm 1066

482

483

484

485 Table 3. Model comparisons by Akaike's Information Criterion (AIC) for survival distributions
 486 of male and female summer-run steelhead handled using electronarcosis, V-board, or unhandled
 487 control within different parametric distributions. Δ AIC is comparison with minimum AIC in
 488 each distribution.

Distribution	Model	df	AIC	Δ AIC
Weibull	Constant	2	2193.88	70.58
	Sex	3	2125.91	2.61
	Treatment	4	2193.94	70.63
	Treatment + Sex	5	2123.30	0.00
	Treatment + Sex + Interaction	8	2126.43	3.12
Exponential	Constant	1	2602.23	49.44
	Sex	2	2552.79	0.00
	Treatment	3	2604.06	51.28
	Treatment + Sex	4	2554.19	1.41
	Treatment + Sex + Interaction	7	2558.25	5.46
Gaussian	Constant	2	2194.71	83.78
	Sex	3	2119.87	8.94
	Treatment	4	2188.47	77.55
	Treatment + Sex	5	2110.93	0.00
	Treatment + Sex + Interaction	8	2112.44	1.51
Logistic	Constant	2	2195.66	79.02
	Sex	3	2122.92	6.27
	Treatment	4	2193.03	76.38
	Treatment + Sex	5	2116.65	0.00
	Treatment + Sex + Interaction	8	2118.26	1.61
Lognormal	Constant	2	2210.81	82.09
	Sex	3	2138.55	9.83
	Treatment	4	2203.67	74.96
	Treatment + Sex	5	2128.72	0.00
	Treatment + Sex + Interaction	8	2130.13	1.42
Loglogistic	Constant	2	2196.26	76.20
	Sex	3	2126.10	6.04
	Treatment	4	2193.71	73.65
	Treatment + Sex	5	2120.06	0.00
	Treatment + Sex + Interaction	8	2121.84	1.78

490
491 Table 4. Model comparisons by Akaike's Information Criterion (AIC) for survival distributions
492 of summer-run steelhead handled using electronarcosis, V-Board, or unhandled control between
493 parametric distributions. The best model chosen from within the five models in each
494 distribution. Δ AIC is comparison with minimum AIC for all distributions.

Distribution	Best Model	df	AIC	Δ AIC
Weibull	Treatment + Sex	5	2123.30	12.38
Exponential	Sex	2	2552.79	441.86
Gaussian	Treatment + Sex	5	2110.93	0.00
Logistic	Treatment + Sex	5	2116.65	5.72
Lognormal	Treatment + Sex	5	2128.72	17.79
Loglogistic	Treatment + Sex	5	2120.06	9.13

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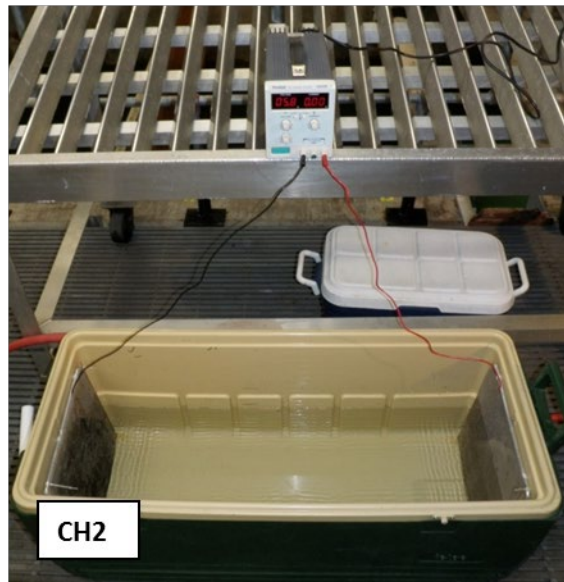
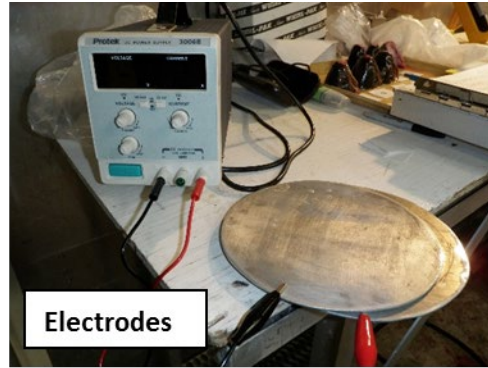
500 Figure 1. V-trough used by WDFW and electronarcosis electrodes and chambers constructed for
501 experiments conducted on summer-run steelhead at LFH.

502

503 Figure 2. Survival distributions of male and female summer-run steelhead handled using V-
504 trough, electronarcosis, and an unhandled control group estimated using a Gaussian model. The
505 dotted lines represent the 95% confidence interval surrounding the survival distribution projected
506 for the unhandled control group.

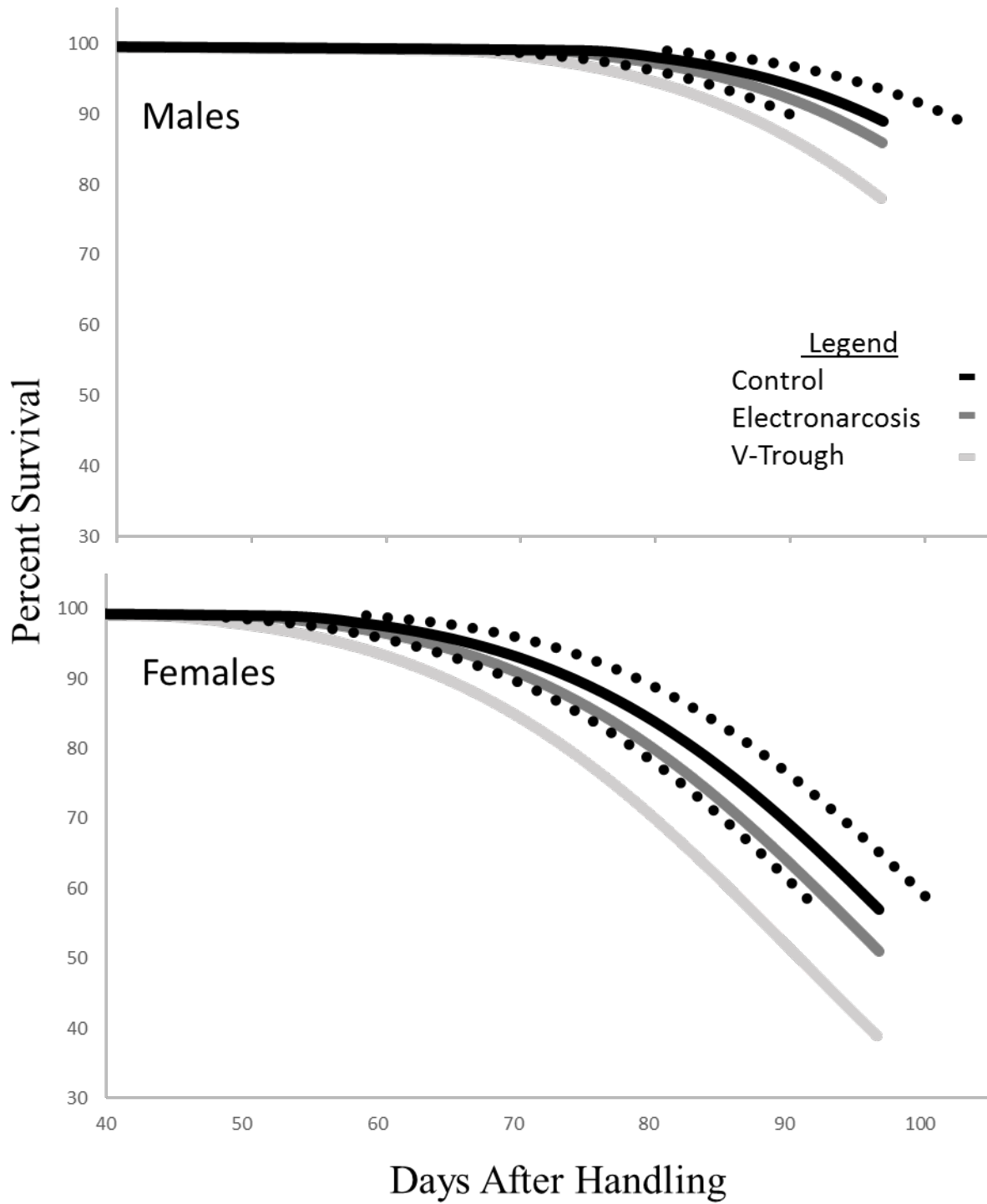
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Figure 1.



514 Figure 2

