Impact of electric fishing on egg survival of whitefish, *Coregonus lavaretus*

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Abstract Previous studies on the effects of electrofishing on fish eggs have reported widely varying results, ranging from gentle to adverse impacts on egg survival rates, and this may be attributable to inadequately standardised assessment methods. The impact of electrofishing on the survival of European whitefish, *Coregonus lavaretus* L., eggs was analysed in laboratory experiments and in a comparative field study. Different anode distances and time points post-treatment were evaluated after artificial fertilisation with milt of untreated males, and survival rates were determined in the morula stage. In the laboratory, no significant difference in survival rates was found between various time points of stripping the gametes post-electric shock. In the treatment group, significantly lower survival rates were detected in eggs (98.6%; SE = 0.20%) compared to the control group (99.6%; SE = 0.11%). Analysis of the egg survival rates in relation to effective voltage, rather than anode distance, revealed a significant trend of declining survival with increasing effective voltage. In field studies, eggs of electroshocked fish showed significantly lower survival (94.8%; SE = 1.03%) than untreated controls (98.7%; SE = 0.38%). Although electrofishing enhanced egg mortality, overall survival was noticeably high (>90%), indicating a relatively small impact.

KEYWORDS: anode distance, broodstock, electric field, gametes, head-to-tail voltage, salmonid.

Introduction

Electric fishing (known widely as ‘electrofishing’) is a highly effective method employed to capture freshwater fishes for non-lethal sampling and measurement, often with the fish released after measurement (Cowx & Lamarque 1990). Other options for the use of electric fishing are fish rescue, selective elimination of species or broodstock capture (Lamarque 1990). As such, electrofishing is considered to be a relatively harmless, low-impact method for stock assessment and for capturing target species. However, there are potential risks, such as improper use of electric equipment (e.g. high voltages or long exposure), which can cause injuries, long-term trauma or even mortality (Lamarque 1990; Beaumont et al. 2002; Reynolds & Kolz 2012). Excessive exposure to high-intensity electrical fields is thought to be one of the major causes for casualties during electrofishing (Snyder 2003). Fish can suffer from haemorrhaging or spinal cord injuries caused by strong muscle contractions or may die due to muscle tetanus and exhaustion (Lamarque 1990), which may appear immediately or even after hours or days (Barnes et al. 1999). For Arctic charr, *Salvelinus alpinus* (L.), exposure to an electric field of 4 V cm⁻¹ increases blood pressure whilst blood oxygen decreases, with specimens experiencing cardiac arrest, ceased ventilatory activity and in some cases systemic hypoxaemia leading to death (Sandblom et al. 2012). Other species have been reported to suffer adverse effects on fish behaviour, health, growth and reproduction, such as reduced reproductive success in nest-guarding male largemouth bass, *Micropterus salmoides* (Lacepède), which did not reoccupy their nests after receiving an electric shock (Siepker et al. 2006). A review of adverse effects on fish reported for different species in several studies is provided by Snyder (2003).

For fish embryos, the reported effects of electricity have indicated that survival is more affected in early development (morula and epiboly stage), and electrofishing over spawning grounds may significantly reduce the survival of embryos (Roach 1999; Bohl et al. 2009). Shocking eggs of Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), for 10 s in the early eyed stage can cause an increase in mortality from 2.1 to 34.2% (Cho et al. 2002).

Whilst the instantaneous risks of injuries and mortality to fish and embryos during electrofishing are well...
studied, there is sparse information on the effect of electrofishing on gametes of sexually mature salmonids. In long-term studies, yearling rainbow trout, Onchorhynchus mykiss (Walbaum), were exposed to a voltage gradient of 1 V cm⁻¹ for 30 s (Maxfield et al. 1971). Compared to a control group, shocked yearlings did not show long-term effects on growth until sexual maturity nor significant differences in the fitness of their offspring. An immediate effect on the fertility of ripe females (spawners) was found when using gametes from shocked (100 V, AC) and unshocked pink salmon, Onchorhynchus gorbuscha (Walbaum), for breeding experiments (Marriott 1973). Milt and eggs from both groups were combined (a subset of egg samples from treated and untreated spawners was inseminated with milt from treated and untreated males), showing that there was no effect of male shocking on egg survival, but shocking of females nearly doubled egg mortality from 15.8 to 27.6%. Eggs of O. tshawytscha caught by electrofishing did not show differences in egg size or number to a control group, but an increased mortality until eyed stage in eggs from electro shocked females (37.9 vs 17.9% in the control group) was observed (Barnes et al. 1999). In another study of O. tshawytscha, high mortalities (39.4%) were reported for eggs from electroshocked females (PDC; 200 V, 80 Hz, 10 s), whereas the mortalities in the control group were 9.9% (Cho et al. 2002). By contrast, decreased mortality was found for eggs of electro-anesthetised O. tshawytscha – 4.8% compared with 10.0% in the control group (Tipping & Gilhuly 1996). For arctic grayling Thymallus arcticus (Pallas), increased mortality of eggs of electroshocked parents was observed (Roach 1999). An additional exposure of the fertilised eggs to different electric field intensities led to significantly higher mortality rates increasing with higher voltage gradients. High-voltage gradients also had negative effects on eggs of chum salmon Oncorhynchus keta (Walbaum). Electrical immobilisation of fish with combinations of voltages above 300 V and currents over 6.0 A led to increased egg mortality from 2.1 to 21.1% up to the eyed stage (Tesch et al. 1999).

There is only sparse data on the effect of electrofishing on egg fertilisation in whitefish Coregonus spp. This may be because Coregonus spp. are typically distributed in deeper waters where they are caught with nets. For the least cisco, Coregonus sardinella Valenciennes, significantly higher egg mortality (59 vs 52%) was found in an electroshocked group compared to a control group, and for electroshocked humpback whitefish Coregonus pidischian (Gmelin), egg mortality rates of 41% were observed (Roach 1996).

The aim of this study was to determine the effects of electric fields on egg survival of sexually mature European whitefish Coregonus lavaretus L. spawners. To obtain reproducible and standardised data under controlled conditions, a laboratory-based experimental approach was used. The results from experiments were then compared with the effects of electrofishing on ripe females caught in the field at their spawning site. This is important to evaluate the use of electrofishing to obtain broodstock for breeding programmes.

Methods

The population of C. lavaretus from Lake Hallstatt, Austria (47°33’ N; 13°40’ E), is genetically characterised as belonging to a native Alpine lineage (Winkler et al. 2011). All fish used in the laboratory experiments were caught with dip nets during their spawning run in the River Koppentraun. In previous years, broodstock fish were caught at the spawning grounds and unripe or overripe fish were never observed. Furthermore, in previous years, ripe fish were successfully kept in captivity for 3 days without negative effects on their egg quality (J. Wanzenböck, unpublished data).

Fish were held in oxygenated fibreglass tanks and transported to the research facility in Mondsee (Austria), where they were held in round tanks with a diameter of 3 m for 1 day before experimentation. All fish were held in filtered, ozonated water pumped from Lake Mondsee throughout the experiments (for details, see Wanzenböck et al. 2012), which used a standard backpack electrofishing generator of 1.3 kW (ELT 60 II GI; max. 300 V DC, max. 7 A; Grassl, Schönau, Germany). For the experimental setup, only standard equipment was used to facilitate repeatability. Both electrodes were made of standard electrofishing cathodes consisting of 2.5-m-long braided copper strips, rolled to dishes of 10 cm in diameter and placed on opposite sides of a round tank of 3 m in diameter. Distances of 10, 40 and 80 cm from the anode were marked at the bottom of the tank before it was filled with water to 15 cm depth (Fig. 1a). The conductivity of the water was 330 µS during experiments.

Female fish were randomly divided into control and treatment groups, which each comprised 15 individuals anaesthetised with tricaine methanesulphonate (MS-222). The two groups were separated into three distance groups (10, 40 and 80 cm from the anode) containing five individuals each. As anaesthetised fish did not show any movement, and their heads (the heaviest body part) were touching the bottom of the shallow tank, only one posterior tether was needed to maintain orientation during the electroshocking procedure. Therefore, lead weights were bound to the caudal peduncle with rubber bands, which adequately prevented passive movement.
towards the anode during the experiment. The mean fish total lengths (TL, cm) within the three distance groups (10, 40 and 80 cm) of the treatment group were 321 mm (SE = 10.1), 323.6 mm (SE = 5.9) and 319 mm (SE = 4.2) and did not differ significantly (P = 0.93). The mean TLs within distance groups of the control were 315 mm (SE = 5.6), 356 mm (SE = 30.1) and 381 mm (SE = 28.9) and did not differ significantly (P = 0.19). Overall, mean fish TL in the treatment group was 322 mm (SE = 4.0), whilst in the control group, it was 348 mm (SE = 14.6), which were significantly different (t-test: P < 0.0001).

For the treatment group, 250 V DC and 0.5 A were applied for 10 s. The control group was handled in the same way but not electroshocked. After the treatment, the females of each group were individually marked by fin clipping. The fish of each group were held separately, and egg samples were collected by partially stripping 10, 30 and 120 min post-treatment, creating three subgroups for the egg collection time (Fig. 1b). At these three time points, each fish was partially stripped into an individual spawning pan, and the egg samples were combined with milt from three randomly chosen males and activated by mixing with water. The mixture was carefully stirred with a feather to enhance fertilisation and reduce the stickiness of the eggs without physical damage. After 3 min, the eggs were washed with water to remove excess sperm. For each individual, three samples of 30 inseminated eggs were taken per time point and incubated in Petri dishes at 6 °C in a professional refrigerator equipped with a fan-powered air distribution system to eliminate any vertical or horizontal temperature

Figure 1. Setup and design of laboratory experiments for testing egg survival in electroshocked whitefish spawners. (a) Fish from both test and control groups were split into three anodal distance groups (10, 40 and 80 cm) and fixed at the bottom of the tank. (b) Female whitefish were divided into two groups (control and treatment groups) with 15 individuals each. Both groups were separated into three distance groups with five individuals each. Subgroups resulted from the partial stripping at different time points post-treatment (10, 30 and 120 min). (c) Fertilised and viable egg in morular stage; developing cells lead to a diffused grayish patch, oil droplets aggregated. Non-viable (probably unfertilised) egg (below): no cell division, opacity is constant in the entire egg; oil droplets spread over yolk.
was determined to calculate survival rates. Viable eggs were identified under a stereo microscope, as eggs showed a change in opacity due to cell division (Fig. 1c; upper egg). Dead or unfertilised eggs were either turning white because of coagulating protein, or stayed clear with oil drops remaining dispersed (Fig. 1c; lower egg). The number of viable eggs was determined to calculate survival rates.

To quantify the exact intensities of fish exposure to the current, 10 additional fish were overanesthetised with MS-222, and measuring electrodes were fixed with cable straps mounted through the opercula and a clip at the end of the tail fin. Those fish were individually exposed to the electric field (250 V DC; 0.5 A; 330 µS), and orientation in the tank was provided by holding the fish with insulating neoprene gloves. The effective head-to-tail voltage and current were measured for each fish with a standard multimeter (Volcraft VC 150, Conrad Electronics, Hirschau, Germany) at distances of 0, 10, 40 and 80 cm from the anode, that is four distance groups as opposed to only three distance groups used with the anesthetised fish. Values for head-to-tail voltage and current were standardised by dividing them by TL to obtain values for the effective voltage and current. Data on survival rates of eggs from anesthetised fish for each group (treatment and control) and for each distance group (10, 40, and 80 cm) were compared between time subgroups (time points of stripping after treatment) by separate one-way ANOVAS.

In the comparative field study, 10 females and 15 males were captured with dip nets at their natural spawning sites. Subsequently, another 10 females were caught by electrofishing as the treatment group (250 V DC, 0.75 A); the mean TL of electroshocked fish was 336 mm (SE = 8.9) and 313 mm (SE = 4.9) for the control fish. Electroshocked and control females were transported separately to Mondsee, and eggs were collected 10 h post-treatment. Each female was stripped individually and the gametes were inseminated as described above with milt from males that have not been electroshocked. From every female fish, three samples of 30 inseminated eggs each were incubated in Petri dishes at 6 °C in a refrigerator. The Petri dishes were inspected after four days, and unfertilised (dead) eggs were counted 8 days after fertilisation.

According to Austrian law and EU directive 2010/63/EU no animal experimentation license was required because all techniques used are established, non-experimental practices in agriculture, i.e. aquaculture and/or fisheries. Statistical analysis was performed using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego CA, USA, www.graphpad.com). Samples were compared using one-way ANOVA (Howell 2002), testing whether or not two or more groups of samples originated from statistical populations with same mean values. In case of non-homogeneous variances, a Kruskal–Wallis H-test (Kruskal & Wallis 1952) was performed. Owing to non-normal distribution of the data of the test and control groups in the laboratory experiment, respectively, the field study was compared by Mann–Whitney U-tests. In cases where no differences were observed, the data were pooled to form only distance groups. Because distance is only a partial proxy for exposure to electric fields, the data set was analysed by assigning the mean survival rate of each treated distance group and the control group (anesthetised fish) to the mean effective voltage measured in the corresponding distance group on dead fish. It was assumed that treated (anesthetised) fish would have experienced similar effective voltages as dead fish within their respective distance group.

Results

In the tank experiment, the mean head-to-tail voltage measured for the 10 dead individuals (mean TL = 32 cm; SD = 1.04) was 56.55 V at the anode and decreased to 14.40 V at a distance of 80 cm (Fig. 2a). As expected, values were highest at the anode and decreased as distance to the anode increased. The mean current reached 59.80 mA at the anode and decreased to 11.85 mA at 80 cm distance. Standardised mean head-to-tail values of 1.79 V cm⁻¹ (SD = 0.10), 1.02 V cm⁻¹ (SD = 0.04), 0.62 V cm⁻¹ (SD = 0.01) and 0.48 V cm⁻¹ (SD = 0.04) were obtained for the four distance groups (see Fig. 2a; details are provided in Table S1).

Comparisons using one-way ANOVA confirmed that there were no significant differences in the survival rates between the time points post-treatment: control group: P = 0.26 (10 cm), 0.22 (40 cm) and 0.09 (80 cm); test group: P = 0.45 (10 cm), 0.41 (40 cm) and 0.62 (80 cm). For the 40- and 80-cm anode distance groups of the test group, there were also no significant differences (Kruskal–Wallis H-tests; P = 0.57 and 0.47, respectively) in survival rates (Figure S1A; details are provided in Table S2).

The survival rates were not significantly different between distance groups (ANOVA), neither in the treatment (P = 0.36) nor in the control group (P = 0.75), indicating that there was no significant effect of the anode distance (see also Figure S1B). In a next step, pooled data (with regard to anode distance and time of stripping) from the control and treatment groups were found to differ significantly (Mann–Whitney U-test; P < 0.0001). The survival rates in the treatment group (98.6%; SE = 0.20) were significantly lower than in the
control group (99.6%; SE = 0.11, see Fig. 3a). Conversely, the egg mortality in the test group (mean 1.4%, SE = 0.20) was higher than the control group (mean 0.4%; SE = 0.11).

As distance is only a partial proxy for exposure to electric fields (see Fig. 2a), a significant ($F$-test; $P = 0.0007$, $r^2 = 0.991$) linear relationship (Fig. 2b) was found, where mean survival rates decreased with increasing effective voltage ($y = 99.66 - 1.45x$).

In the comparative field study, the mean survival rate of eggs for unshocked females was significantly (Mann–Whitney $U$-test) higher (98.7%; SE = 0.38) than that (94.7%; SE = 1.03) for the shocked females ($P = 0.0007$). The mortality rates from eggs in the field study were 1.3% (SE = 0.38) in the control group compared with 5.2% (SE = 1.03) in the treatment group; this highlights that electrofishing caused a fourfold higher percentage of non-viable (possibly unfertilised) eggs than netting (Fig. 3b; details are provided in Table S3).

**Discussion**

In the present study, survival was found to be significantly reduced in eggs of electroshocked spawners, which is roughly comparable to the findings for *C. sardinella* (Table 1). The percentages of viable eggs were clearly higher in the present study, but nonetheless, the adverse effect of electroshocking was confirmed for *C. lavaretus*. Most surveys depict an increase in egg mortality due to electric shocking between 100 and 200% (Marriott 1973; Dwyer et al. 1993; Roach 1996; Redman et al. 1998). The findings of the present study are somewhat higher (a fourfold increase), but in one case, extreme mortalities of up to 18-fold higher (Roach 1996) were observed. In another exceptional study, contrasting findings with a higher survival compared with the control group were reported (Tipping & Gilhuly 1996).

The variation between the species may be explained by different sensitivities to the electric field. According to the literature (Lamarque 1990; Beaumont *et al.*...
Table 1. Mortality in percentage (%) and percentage change (Δ %) in fish eggs from electroshocked parents found in various studies of Coregonus sardinella Valenciennes, C. pidschian (Gimelin), C. lavaretus (Linnaeus), Oncorhynchus tshawytscha (Walbaum), O. mykiss (Walbaum), Salmo trutta Linnaeus, O. clarkii (Richardson), Thymallus arcticus (Pallas), Esox lucius Linnaeus, Xyrauchen texanus (Abbott)

<table>
<thead>
<tr>
<th>Species</th>
<th>Control</th>
<th>Test</th>
<th>Δ %</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
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<td>56.8</td>
<td>4.8</td>
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</tr>
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<td>1.4</td>
<td>1.0</td>
<td>Present study (laboratory)</td>
</tr>
<tr>
<td>Coregonus lavaretus</td>
<td>1.3</td>
<td>5.3</td>
<td>4.0</td>
<td>Present study (field)</td>
</tr>
<tr>
<td>Oncorhynchus tshawytscha</td>
<td>1.2</td>
<td>20.0</td>
<td>18.8</td>
<td>Roach (1996)</td>
</tr>
<tr>
<td>Oncorhynchus tshawytscha</td>
<td>17.9</td>
<td>37.9</td>
<td>20.0</td>
<td>Barnes et al. (1999)</td>
</tr>
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<td>6.8</td>
<td>2.4</td>
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<td>Oncorhynchus tshawytscha</td>
<td>11.8</td>
<td>6.6</td>
<td>–5.2</td>
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</tr>
<tr>
<td>Oncorhynchus mykiss</td>
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<td>57.9</td>
<td>28.1</td>
<td>Dwyer et al. (1993)</td>
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<td>27.6</td>
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<td>27.0</td>
<td>4.0</td>
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<td>Salmo trutta age 3 years</td>
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<td>Oncorhynchus clarkii</td>
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</tr>
<tr>
<td>Xyrauchen texanus</td>
<td>89.0</td>
<td>95.0</td>
<td>6.0</td>
<td>Muth and Ruppert (1996)</td>
</tr>
</tbody>
</table>

Bold values highlight the findings of the present study.

2002), salmoniform species are more influenced by electrofishing than others; this also seems to be the case for their gametes. Surveys for northern pike (Walker 1999) reported less pronounced increases in egg mortality (not more than 2.3%) than that (6.7%) observed (Abbott) reported less pronounced increases in egg mortality. The decrease in mortality due to electrofishing stands contrary to all other studies and previous experiments (Roach 1996; Tipping & Gilhuly 1996; Barnes et al. 1999; Zydlewski et al. 2008) vary from –66 to 1670% changes in mortality. The decrease in mortality due to electric fishing stands contrary to all other studies and the results of the current experiments. In some studies, higher egg mortalities were found for electrofishing with voltages between 250 and 350 V (Roach 1996; Barnes et al. 1999) than for electric immobilising with high voltage up to 550 V (Tipping & Gilhuly 1996; Zydlewski et al. 2008). These contrasting reports illustrate the necessity to obtain more comparable information about the actual voltages experienced by the fish. To obtain comparable data, a standardised method is required. One such method may be the experimental setup developed for laboratory experiments in the present study (Fig. 1a). In comparison, the results of the laboratory experiments and field study closely match, with reductions in survival differing ≤15%. It therefore seems possible to estimate the impact in the field based on data obtained from laboratory experiments. The advantages of this experimental setup include repeatability and relatively low costs because only standard equipment was used. In future investigations, this setting may be used to study the impact of electric exposure on different species, fish size and/or fish numbers under defined conditions. It may be of interest to evaluate different water characteristics such as conductivity, temperature, pH or oxygen, which can be adjusted easily in the laboratory. If slightly modified using a generator with adjustable power output, the setup will also be suitable to evaluate threshold levels for field intensities. The use of this setting as a standard will produce comparable data, which may help to optimise electrofishing practice.

Owing to the high survival rates (≥90%) of C. lavaretus found in this study, and previous experience (J. Wanzenböck, unpublished data), overripening does not seem to be an issue, although in a study on closely related C. sardinella and C. pidschian (Roach 1996), problems were encountered to catch enough fish. This may be indicative of the end of the spawning season, and lower survival rates (∼50%) were reported, which may have occurred due to overripening. The overripening of eggs has been identified as a limiting factor in spawning success (Mohagheghi Samarin et al. 2010), with the suboptimal quality of overripened eggs
responsible for decreased fertilisation and abnormalities in the development of the embryo (Lahnsteiner 2000). In further studies, good timing (i.e. exact meeting of the spawning season) will be essential.

One mechanism for the reduced fertilisation in the test groups may be egg activation due to stimulation by the exposition to the electric field. As shown for unfertilised eggs of Japanese rice fish *Oryzias latipes* (Temminck & Schlegel) sustained in isotonic Ringer’s solution, a voltage gradient above 2 V cm⁻¹ leads to separation of the chorion (Yamamoto 1949). As Salmonidae are considered to be sensitive to electric exposure (Beaumont et al. 2002), this effect may even appear at voltage gradients lower than 2 V cm⁻¹. In the present case, electric fishing could have led to activation in the coelomic cavity, so that the micropyle of the unfertilised eggs may have been closed before milt and water were added. Other possible reasons for the decrease in fertilisation of the test groups may be related to stress due to electric fishing. In a study on adult *Salvelinus alpinus* (Sandblom et al. 2012), signs of systemic stress due to exposure in an electric field were observed, including increased blood pressure, altered hydromineral balance and temporary cardiac arrest. Increased levels of Na⁺, K⁺ and Ca²⁺ in the blood stream (Sandblom et al. 2012) may also lead to an adverse effect on the fertilisation rate and viability of eggs. An alteration in Ca²⁺ levels plays a major role in egg activation-related mechanisms and may be triggered by electric stimulation. For example, a polyspermy block (Iwao 2000) is caused by a shift in the egg membrane potential and leads to an inhibition of sperm entry into the egg. Temporary cardiac arrest in adult fish may also lead to hypoxia (Sandblom et al. 2012); owing to this lack of oxygen, the pH of the ovarian fluid may decrease. Lowered pH in the ovarian fluid is correlated with a significant loss in fertilisation capacity, as it has an adverse effect on sperm motility in Salmonidae (Tabrizi et al. 2011). As pH was not determined in this study, it remains a topic for further research.

In the light of the present study, electrofishing for broodstock may have minimal adverse effects on the reproduction of *C. lavaretus* if conducted properly. In the current experiments, the reduction in the viability of eggs due to electrofishing was significant, but the magnitude of the adverse effect in the laboratory and in the field was low and arguably without practical relevance. It is therefore possible to use electric fishing to catch broodstock without risking high mortalities. In general, the adverse effect of electrofishing on the fertilisation rate and viability of eggs in mature ripe fish is low and may be neglected when working with common species and large populations. To reduce the potential risks of electric fishing (e.g. stress, haematoma, spinal injuries), proper use of the equipment and careful fish handling are imperative.

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References


Electrofishing Impacts Whitefish Egg Survival


Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Survival rates for *C. lavaretus* at different time points post treatment and anode distances from the laboratory experiment.

**Table S1.** Effective head-to-tail (h–t) voltage (V, in V cm⁻¹) and current (in mA) for *C. lavaretus* at different anode distances (in cm).

**Table S2.** Egg survival rates for *C. lavaretus* in the control and test groups of laboratory experiments.

**Table S3.** Egg survival rates for *C. lavaretus* in the control and test groups of the field study.