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[Article]

# Three-Pass Depletion Sampling Accuracy of Two Electric Fields for Estimating Trout Abundance in a Low-Conductivity Stream with Limited Habitat Complexity

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*Abstract*.—We evaluated three-pass depletion sampling for both AC and pulsed-DC electrofishing for estimating the population size of rainbow trout *Oncorhynchus mykiss* in a representative low-conductivity (20-μS/cm) southern Appalachian stream with limited habitat complexity. Trout capture efficiencies in such streams could be expected to exceed those observed in streams in which habitat is more complex; thus, depletion estimates could be much more accurate in the former. We also compared the results for two trout length-groups to investigate size-related differences. Measured capture efficiency was 0.88 ± 0.04 (95% confidence interval) for trout greater than 100 mm (typically adults) and 0.65 ± 0.09 for trout less than 100 mm (age 0). Population size was underestimated in each depletion sample. The errors for trout over 100 mm were generally small (mean, 12%; range, 3–23%), and the upper 95% confidence limits were usually within 10% of the true population size (*N*). Underestimates of *N* were larger for trout under 100 mm (mean, 32%; range, 5–60%), although the upper 95% confidence limits were within 20% of the *N* for half of the samples. The results of a laboratory study confirmed that trout over 100 mm were immobilized at significantly lower voltage gradients than were smaller trout in both electric fields. We conclude that three-pass depletion sampling is relatively accurate in typical southern Appalachian trout streams and that the underestimation errors for rainbow trout larger than 100 mm would be acceptable given basic inventory and monitoring goals.

Trout populations in small (first-order through third-order) southern Appalachian streams are often sampled with backpack electrofishing gear and depletion sampling techniques to obtain abundance estimates (Neves and Pardue 1983; Ensign et al. 1991; Habera et al. 1996). These sampling efforts are typically part of inventory and monitoring programs and provide information that serves as the basis for managing wild (i.e., self-sustaining) trout fisheries throughout the region. For other stream-dwelling salmonid populations, depletion sampling has been widely used to evaluate management and land-use practices (Platts and Nelson 1988; Paul et al. 2003), spatial and temporal variation (Kocovsky and Carline 2006; Dauwalter et al. 2009), habitat restoration structures (House 1996), and conservation status (Zoellick et al. 2005; Meyer et al. 2006a). It also provides the best alternative for obtaining abundance estimates for small nongame fishes, which are not well suited to mark–recapture techniques (Simonson and Lyons 1995). Many larger southern Appalachian trout streams are inhabited by populations of various native cyprinids, catostomids, cottids, and percids, which are also of interest to managers; thus, removal depletion sampling techniques are commonly used.

Several studies have challenged the reliability of abundance estimates obtained by depletion sampling because the constant-catchability assumption of the removal model is frequently violated (Peterson and Cederholm 1984; Riley and Fausch 1992; Peterson et al. 2004; Rosenberger and Dunham 2005). In such cases, decreasing catchability over iterative removal passes results in underestimated population sizes and overestimated capture efficiency (Peterson et al. 2004; Rosenberger and Dunham 2005). Peterson et al. (2004) found that three-pass depletion sampling overestimated capture efficiency by 39% and underestimated abundance by 88%. However, our observations suggest that

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these biases are less severe in many southern Appalachian trout streams, which are characterized by low-conductivity and limited habitat complexity. A clearer understanding of the actual bias associated with depletion sampling in a typical southern Appalachian stream will help fishery managers select appropriate sampling techniques and interpret data properly, which will ultimately facilitate better management decisions based on abundance information.

Electrofishing in waters of exceptionally low conductivity ($\leq$20 μS/cm), such as many trout streams in the southern Appalachian Mountains, requires high voltage ($>$500 V) and workers historically selected alternating current (AC) waveforms to effectively capture fish (Lennon and Parker 1957; Seehorn 1968; Habera and Strange 1993). Contemporary DC or pulsed-DC (PDC) gear lacked power sources capable of producing power densities high enough to immobilize trout and generally were not used unless water conductivity was increased with salt (Gatz et al. 1986; Cada et al. 1987). Consequently, the use of AC electrofishing gear predominated throughout the region until recently.

The primary objective of our study was to evaluate the accuracy of depletion sampling of rainbow trout *Oncorhynchus mykiss* in a small southern Appalachian trout stream with limited habitat complexity. Both AC and PDC backpack electrofishing gear were used for depletion sampling to investigate the comparability of their respective electric fields and determine capture efficiencies and the level of negative bias associated with the corresponding population estimates (stratified by fish size). The newer generation of PDC gear (e.g., Smith-Root's LR-24 backpack units) is capable of producing more powerful electrical fields, and some natural resource management agencies are now using it to sample low-conductivity streams in the region. A controlled laboratory experiment (using fish from the stream) was also conducted to examine the effect of fish size with respect to voltage gradient and electrode proximity necessary for immobilization in low-conductivity water.

## Study Site

Depletion sampling assessments were conducted with rainbow trout in Roaring Fork, a third-order stream in Great Smoky Mountains National Park (35.69724°N, 83.47066°W). Roaring Fork's conductivity (20 μS/cm), alkalinity (15 mg/L as $CaCO_3$), and pH (6.8) are typical of many southern Appalachian trout streams. It also has a relatively abundant wild (i.e., naturalized) rainbow trout and was readily accessible by means of an adjacent road.

Electrofishing was conducted on 9 and 10 August 2006 in a 370-m stream section partitioned with block nets (6–10-mm bar mesh) into four sample sites (40–57 m) separated by buffer zones (25 m, 116 m, and 25 m). Flow was stable at 0.1 $m^3/s$, and temperatures ranged from 18.3°C to 18.7°C. Gradient for this portion of Roaring Fork was 8%, pool : riffle ratios in the sample sites were approximately 1:1, and pool depths were less than 1 m, all of which promote effective electrofishing (Habera et al. 1992). Substrate consisted of cobble, gravel, and boulders, with smaller amounts of sand, silt, and bedrock in some pools. Complex habitat (e.g., large woody debris accumulations, undercut banks; Thompson and Rahel 1996; Peterson et al. 2004; Rosenberger and Dunham 2005) that could reduce capture efficiency was not present. A lack of large woody debris is typical of many southern Appalachian streams in second-growth forests (Silsbee and Larson 1983; Flebbe and Dolloff 1995). Mean channel width for the four sites ranged from 5.3 to 8.2 m; thus, two electrofishing units were used to ensure effective sampling (Habera et al. 1992).

## Methods

*Electrofishing gear*.—Electrofishing gear consisted of the National Park Service's gasoline-powered AC units (not commercially available) and Smith Root, Inc.'s (Vancouver, Washington) model LR-24 DC units. Each AC electrofisher used a 60-Hz generator and transformer and was capable of producing outputs of 100–700 $V_{rms}$ (Habera et al. 1996). The AC units had two hand-held electrodes (30 × 32 cm), one of which was and fitted with a 4-mm-mesh nylon net (Habera et al. 1996) to enable the operator to capture fish. The PDC unit had a hand-held, ring-shaped anode (28 cm in diameter) and a "rattail" cathode (2.8 m long) that was dragged along the streambed behind the operator, who also carried a dip net similar to those used as AC electrodes to equalized the fish capture capability of each system.

Fish immobilization is the standard endpoint in electrofishing studies examining the power transfer theory (Kolz and Reynolds 1989; Miranda and Dolan 2003; Bearlin et al. 2008). The AC units in this study were operated at 600 $V_{rms}$, which is within the range typically used in Tennessee streams where conductivity is 20 μS/cm or less (Moore et al. 1983; Habera et al. 1992, 1996). This level has proven to adequately immobilize rainbow trout in Roaring Fork. Trout immobilization settings for the PDC units were determined by preliminary onsite testing. These units had an autosetting capability that was checked (830 $V_{peak}$, 30 Hz, 12% duty cycle) but did not adequately immobilize fish for capture. Therefore, PDC units were set to the AC output frequency (60 Hz), and voltage and duty cycle were then manually adjusted until fish immobilization was similar to that for AC. This occurred at 990 $V_{peak}$ and 20% duty cycle, with a rectangular 3.3-ms DC pulse waveform that produced limited galvanotaxis during electrofishing. A duty cycle exceeding 10% was preferred to minimize fish injury (Dolan and Miranda 2003, 2004; Miranda and Dolan 2004). Other voltage and duty cycle combinations could also have immobilized trout in Roaring Fork. Immobilization is a function of power transferred from water to fish, and power is the product of voltage and amperage, which increases with increasing duty cycle.

*Electric field measurement*.—The instream electric field intensity for a representative electrofishing unit of each type was measured in four orientations (right, front, left, and back) relative to the right-hand electrode, which was held in the same position for all measurements. The cathode of the PDC unit trailed behind the operator, and its tip was 2.5 m from the anode. The left-hand electrode of the AC unit was held 1.37 m from the right-hand electrode. Horizontal and vertical voltage gradients were measured with a two-channel oscilloscope (Henry et al. 2003) at 16, 25, 50, and 100 cm from the electrode center for each orientation. Measurement locations were held constant using a prepositioned grid. Measurements were made 15 cm from the water surface, and total water depth was about 30 cm. Voltage gradient vectors in the AC ($V_{rms}$/cm) and PDC ($V_{peak}$/cm) fields were later computed for each location (Henry et al. 2003).

*Experimental technique*.—Sixteen three-pass depletion electrofishing efforts (replications) were made through the four sample sites (two efforts/gear/site) with known numbers of trout from two size-groups in place before each replication (30 fish >100 mm and 20 fish <100 mm). These size-groups generally correspond to adult and age-0 trout, and the stocking rates provided densities common to wild trout streams in the region (10–24 fish/100 $m^2$). Rainbow trout were collected from within the sample sites and elsewhere in Roaring Fork (using AC gear) to provide the necessary supply of experimental fish (480 fish >100 mm and 320 fish <100 mm). Experimental fish were collected the day before the electrofishing gear comparison, separated into the two size-groups (actual sizes, 38–98 and 104–241 mm), and held overnight in live cages. Block nets were placed the following day, and two crews (each consisting of two electrofisher operators, two netters, and two bucket carriers) then conducted the three-pass depletions, following the procedure outlined in Table 1. All electrofishing passes were made in the upstream direction. For consistency, electrofishing operators and netters maintained their duties throughout the study, and the same level of effort was used with each gear. Electrofishing technique was aggressive, whereby electrodes were manipulated as necessary to effectively electrify all potential fish cover.

TABLE 1.—Experimental procedure for conducting three-pass depletion samples, as applied to estimating trout abundance in a low-conductivity southern Appalachian stream.

| Step | Procedure |
|---|---|
| 1 | A random set of experimental fish (30 fish >100 mm and 20 fish <100 mm) was tranquilized, marked, allowed to recover, and distributed throughout site 1. |
| 2 | Three electrofishing passes were made through site 1 by crew 1 with AC gear (representing one replication); all captured fish in each size group (>100 or <100 mm) were examined for marks (including those from any previous replications) and counted after each pass. |
| 3 | Steps 1 and 2 were repeated at site 2 with pulsed-DC gear. |
| 4 | Steps 1–3 were repeated, except that the gear types used in each site were switched (i.e., pulsed DC was used at site 1 and AC at site 2). |
| 5 | Crew 2 followed the same procedure (steps 1–4) at sites 3 and 4. |
| 6 | Steps 1–5 were repeated the following day with the electrofishing crews switching sites (i.e., crew 1 worked sites 3 and 4 and crew 2 worked sites 1 and 2), producing a total of 16 three-pass replications (two/gear/site). |

Each set of experimental fish (i.e., the fish used for a particular three-pass replication) was anesthetized and uniquely marked before use. Four sets of fish were required for each site; thus, four fin clips were used: adipose, left and right pectoral tips, and upper caudal lobe tip. Elapsed time between the release of experimental fish for a particular replication and the beginning of electrofishing for that replication was 1–2 h. Elapsed time between successive electrofishing passes was about 0.5 h (Bohlin et al. 1989; Peterson et al. 2004). All fish captured during each electrofishing pass were held in cages in the buffer zones after processing.

*Laboratory fish immobilization assessments*.—Because previous research (e.g., Dolan and Miranda 2003) and our own field experience suggested that trout size affects the voltage gradient necessary for immobilization, we examined this relationship in the laboratory in August 2007. Those findings, in combination with the onsite electric field measurements, made it possible to determine electrode proximity necessary for full immobilization. Additional rainbow trout were collected from Roaring Fork using AC electrofishing gear and held in instream cages for 24 h before these experiments. Fish were transported to the laboratory and held in 200-L plastic tanks containing stream water for less than 30 min before use in exposure tests. Two identical 40-L glass aquaria containing 20 L of stream water (21.0–22.7°C, conductivity 28–31 μS/cm) were used as exposure chambers. Each aquarium was fitted with cross-sectional aluminum plate electrodes at each end (separated by 49 cm) to generate homogenous electric fields within the tank. The homogeneity of the electric field in the exposure tank was verified with the voltage gradient measurement probe (described above) connected to the oscilloscope. Plate electrodes were connected to a backpack electrofishing unit, and a rheostat was placed in the circuit to enable fine adjustments of voltage level. Voltage ($V_{rms}$), voltage amplitude ($V_{peak}$), pulse frequency, and pulse width were measured with an oscilloscope (model THS 720A, Tektronix, Beaverton, Oregon) connected between the plate electrodes of each tank and recorded during each exposure.

For each exposure, a fish was placed in an aquarium and allowed to acclimate for 2–3 min, an electric field was applied for 20 s, and the fish was observed to determine if it was immobilized and whether immobilization occurred within 5 s. Fish were always aligned perpendicular to the plate electrodes when the electric field was applied, although they did not always face the same electrode. Immobilization was defined as the fish having lost all swimming movements and typically included fish losing normal upright swimming posture. Immobilization within 5 s was selected as a relevant endpoint that could be related to the ability to capture the fish in a stream electrofishing situation (i.e., fish would probably escape from the field before capture if immobilization required more than 5 s). Fish were exposed to an electric field only once, and the exposure was to AC or 60-Hz PDC (20% duty cycle, rectangular 3.3-ms pulse) at a specific voltage gradient. Total length of each fish was measured (mm) following exposure.

*Data analysis*.—Measured capture efficiencies were calculated for each trout size-group (<100 mm and >100 mm) as the proportion of true population size (*N*) recaptured after each three-pass replication (eight/size-group by gear type). The two size-groups were examined separately in all analyses because small trout tend to have lower catchabilities (Lohr and West 1992; Anderson 1995; Thompson and Rahel 1996; Korman et al. 2009). Population estimates and 95% confidence intervals (CIs) for each three-pass depletion replication, along with estimated capture efficiencies, were generated with Microfish 3.0 (Van Deventer and Platts 1989) using the maximum-likelihood model (Platts et al. 1983). Microfish also performed chi-square tests to check goodness-of-fit for each removal estimate. Confidence intervals were examined to determine their inclusion of *N* (30 for trout >100 mm; 20 for trout <100 mm). Additionally, capture efficiencies estimated by Microfish 3.0 were compared with measured capture efficiencies for each size-group via a two-sample *t*-test and the NCSS software system (Hintze 2001). Data were checked for normality before statistical testing and significance set at $\alpha = 0.05$.

A logistic model of electroshock-induced immobilization within 5 s of exposure (binary data) was developed based on the effect of voltage gradient (continuous variable) for each waveform and fish-size combination (class variable). Interactions between class variable and voltage gradient were also assessed and included in the model if they were significant ($P < 0.05$) based on the Wald $\chi^2$ statistic. The logistic probability of a fish being immobilized, logit(*P*), was modeled by logistic regression (PROC GENMOD; SAS version 9.1; SAS Institute, Cary, North Carolina). The estimate of logit(*P*) from logistic models was used to obtain the predicted probability of fish immobilization $i = e^{\text{logit}(P)}/(1 + e^{\text{logit}(P)})$. Differences in the ability of each waveform to immobilize each size-group of fish were evaluated in SAS (ESTIMATE statement in PROC GENMOD), where $\alpha = 0.05$ for the pairwise comparisons.

## Results

Voltage gradient in the AC electric field was highest (13.8 $V_{rms}$/cm) at 16 cm in front of the anode and decreased exponentially to 0.7 $V_{rms}$/cm at 1 m (Figure 1). The PDC electric field had similar characteristics in front of the anode (Figure 1). Instream electric field intensities reported here are consistent with those reported for other electrofishing equipment (Henry et al. 2003), but results should be interpreted cautiously because the relative distance between the handheld electrodes changes during backpack electrofishing.

Although no three-pass replication resulted in complete recovery of its corresponding set of marked fish, recapture rates were relatively high. Mean measured capture efficiency for rainbow trout over 100 mm was 0.88 (95% CI, ±0.04) over all three passes. Results for the AC gear (mean, 0.88; range, 0.77–0.93) and PDC gear (mean, 0.87; range, 0.77–0.97) were similar (Table 2). Measured capture efficiency for rainbow trout under 100 mm was lower on average (mean, 0.65) and less consistent (95% CI, ±0.09). The two electric fields also produced similar results for this size-group, measured capture efficiency averaging 0.69 for AC (range, 0.50–0.85) and 0.61 for PDC (range, 0.40–0.95). First-pass capture efficiency was also relatively high for larger trout (mean, 0.74; 95% CI, ±0.05), but was below 50% for smaller fish (mean, 0.46; 95% CI, ±0.09). Despite as many as nine additional electrofishing passes through a given site, only 1 of 32 sets of sample fish was completely recovered (age-0; four passes). A few trout under 100 mm were recaptured after 8, 9, or 10 electrofishing passes, but no trout over 100 mm were recaptured beyond a fifth pass. Additionally, there were no significant differences between the estimated (from Microfish 3.0) and measured capture efficiency for rainbow trout under 100 mm ($t = 1.172$, df $= 15$, $P = 0.251$) or over 100 mm ($t = -1.327$, df $= 15$, $P = 0.194$).

Because both gears produced steep depletion patterns for trout over 100 mm, population estimates equaled the total number of recaptures in all but one case (Table 2). Additionally, the 95% CIs were narrow (typically two or fewer fish). Consequently, the 95% CIs included *N* (30) in only four cases. Although each population estimate was an underestimate of *N*, errors were relatively small (range, 3–23%; mean, 12%) and the upper 95% confidence limits were always within 20% of *N* and often within 10%. Additionally, chi-square test results indicated a lack-of-fit (i.e., unequal capture probability among passes) for only one depletion estimate for trout over 100 mm.

Depletion patterns for trout under 100 mm were more variable than those for larger fish and included three cases where more trout were captured on the third pass than on the second (Table 3). However, most population estimates equaled the total number of recaptures, and the upper 95% confidence limits were

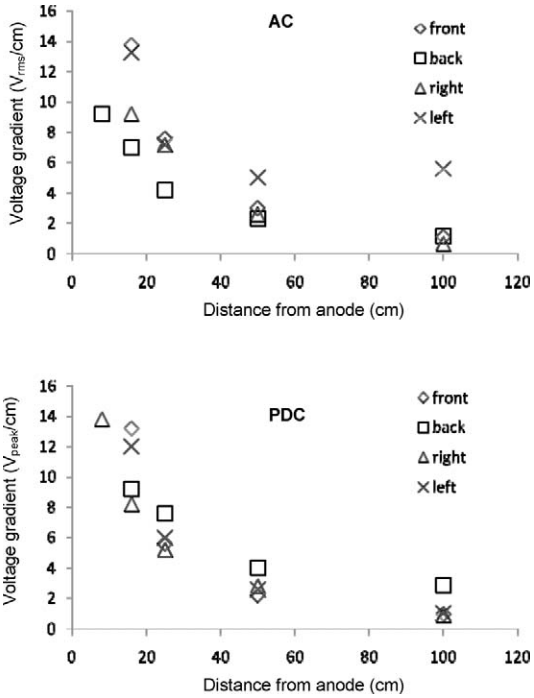

FIGURE 1.—Instream electric field intensities generated by AC (upper panel) and pulsed-DC (lower panel) backpack electrofishing equipment at specific locations relative to the electrodes in a low-conductivity southern Appalachian stream. The measurement points were located in a horizontal line defined by a grid oriented in front, back, left, and right of the anode.

TABLE 2.—Electrofishing and population estimation results for rainbow trout 100 mm or more in length from each replication.

| Site | Depletion pattern[a] | Recaptures | Measured capture efficiency | Population estimate[b] | 95% confidence interval[c] | Estimated capture efficiency |
|---|---|---|---|---|---|---|
| | | | **AC** | | | |
| 1 | 18–5–0 | 23 | 0.77 | 23 | 23–24 (80) | 0.82 |
| | 23–1–0 | 24 | 0.80 | 24 | 24–24 (80) | 0.96 |
| 2 | 24–4–0 | 28 | 0.93 | 28 | 28–29 (97) | 0.88 |
| | 24–1–2[d] | 27 | 0.90 | 27 | 27–28 (93) | 0.84 |
| 3 | 19–6–3 | 28 | 0.93 | 28 | 28–30 | 0.87 |
| | 22–4–0 | 26 | 0.87 | 26 | 26–27 (90) | 0.70 |
| 4 | 25–3–0 | 28 | 0.93 | 28 | 28–28 (93) | 0.90 |
| | 26–1–1 | 28 | 0.93 | 28 | 28–28 (93) | 0.90 |
| | | | **Pulsed DC** | | | |
| 1 | 22–3–3 | 28 | 0.93 | 28 | 28–30 | 0.76 |
| | 25–3–1 | 29 | 0.97 | 29 | 29–30 | 0.85 |
| 2 | 15–4–4 | 23 | 0.77 | 24 | 23–28 (93) | 0.61 |
| | 23–1–0 | 24 | 0.80 | 24 | 24–24 (80) | 0.96 |
| 3 | 20–5–0 | 25 | 0.83 | 25 | 25–26 (87) | 0.83 |
| | 23–3–0 | 26 | 0.87 | 26 | 26–26 (87) | 0.90 |
| 4 | 23–1–1 | 25 | 0.83 | 25 | 25–25 (83) | 0.89 |
| | 21–6–2 | 29 | 0.97 | 29 | 29–31 | 0.74 |

[a] First through third passes.

[b] The true population size was 30 in each case.

[c] The calculated lower confidence limit was less than the number of recaptures in each case; therefore, the lower confidence limits were set equal to the number of recaptures. The values in parentheses are the percentages of the true population size represented by the upper confidence limits.

[d] A chi-square test (calculated value = 5.654) indicated lack of fit.

TABLE 3.—Electrofishing and population estimation results for rainbow trout less than 100 mm from each replication. See Table 2 for additional details.

| Site | Depletion pattern[a] | Recaptures | Measured capture efficiency | Population estimate[b] | 95% confidence interval | Estimated capture efficiency |
|---|---|---|---|---|---|---|
| | | | **AC** | | | |
| 1 | 10–1–2 | 13 | 0.65 | 13 | 13–14 (70) | 0.72 |
| | 12–4–1 | 17 | 0.85 | 17 | 17–18 (90) | 0.74 |
| 2 | 11–2–0 | 13 | 0.65 | 13 | 13–13 (65) | 0.87 |
| | 12–2–1 | 15 | 0.75 | 15 | 15–16 (80) | 0.79 |
| 3 | 5–3–4 | 12 | 0.60 | 19 | 12–47 | 0.27 |
| | 12–2–0 | 14 | 0.70 | 14 | 14–14 (70) | 0.88 |
| 4 | 9–1–0 | 10 | 0.50 | 10 | 10–10 (50) | 0.91 |
| | 10–2–4 | 16 | 0.80 | 17 | 16–22 | 0.55 |
| | | | **Pulsed DC** | | | |
| 1 | 9–0–0 | 9 | 0.45 | 9[c] | 9–9 (45) | 1.00 |
| | 16–2–1 | 19 | 0.95 | 19 | 19–20 | 0.83 |
| 2 | 7–3–2 | 12 | 0.60 | 12 | 12–15 (75) | 0.63 |
| | 10–5–0 | 15 | 0.75 | 15 | 15–16 (80) | 0.75 |
| 3 | 8–1–0 | 9 | 0.45 | 9 | 9–9 (45) | 0.90 |
| | 5–2–1 | 8 | 0.40 | 8 | 8–10 (50) | 0.67 |
| 4 | 2–6–1[d] | 9 | 0.45 | 12 | 9–26 | 0.35 |
| | 10–5–1 | 16 | 0.80 | 16 | 16–18 (90) | 0.70 |

[a] First through third passes.

[b] The true population size was 20 in each case.

[c] A maximum-likelihood estimate was not possible (all fish were caught on the first pass).

[d] A chi-square test (calculated value = 5.441) indicated lack of fit.

typically five or fewer fish above the population estimate. There were again only four cases where 95% CIs included *N* (20). Additionally, underestimates of *N* (range, 5–60%; mean, 32%) were typically more substantial than those observed for larger trout. Upper 95% confidence limits were within 20% of *N* for half of the replications (Table 2). Chi-square tests indicated that only one depletion estimate for trout under 100 mm exhibited lack-of-fit (Table 3).

Immobilization of both trout size groups increased with voltage gradient for both waveforms tested in the laboratory (Figure 2). The class variable (electric field type and fish size) and the interaction of the class variable and voltage gradient were the significant terms in the model. Larger trout were immobilized at significantly lower voltage gradients ($P < 0.03$) than smaller trout in both electric fields. The voltage gradient required to induce 50% immobilization ($IV_{50}$) in trout over 100 mm was 0.77 $V_{rms}$/cm for AC and 1.45 $V_{peak}$/cm for PDC. The $IV_{50}$ for trout under 100 mm was 1.73 $V_{rms}$/cm for AC and 2.23 $V_{peak}$/cm for PDC. The model for trout under 100 mm exposed to AC is shifted right because three fish exposed to 2.0–2.5 $V_{rms}$/cm were not immobilized within 5 s (Figure 1). These three fish were immobilized 6 and 7 s after exposure was initiated. Complete (100%) immobilization of trout over 100 mm occurred at about 1.0 $V_{rms}$/cm in the AC field and at about 1.9 $V_{peak}$/cm in the PDC field. This voltage gradient generally occurred at about 80 cm from the anode in each electric field (Figure 2). Trout under 100 mm were completely immobilized at about 3.8 $V_{rms}$/cm in the AC field and at about 3.3 $V_{peak}$/cm in the PDC field. These voltage gradients occurred much closer (about 40 cm) to the anode in each electric field (Figure 2).

## Discussion

Depletion sampling and the removal model have been widely used to estimate salmonid abundance in small streams. The model assumes a closed population, an equal chance of capture for all individuals, and constant capture probability over all efforts (Zippin 1958), the third assumption potentially being the most difficult to meet. Violation of the constant catchability model assumption has been shown to occur and can produce negatively biased abundance estimates and overestimated sampling efficiencies (Cross and Stott 1975; Riley and Fausch 1992; Peterson et al. 2004; Rosenberger and Dunham 2005). Consequently, uncorrected removal depletion sampling data (and abundance estimates derived from these data) may be unreliable (Peterson et al. 2004; Rosenberger and Dunham 2005; Thurow et al. 2006).

The assumption of equal capture efficiency among passes, however, is critical only when it ranges from 0.2 to 0.5 for three-pass efforts; it is much less important when most fish in the sample population are eventually caught (White et al. 1982; Armour et al. 1983). Rosenberger and Dunham (2005) also conceded that removal estimate bias may be a lesser concern with high sampling efficiency (≥80%). Sweka et al. (2006)

found that mean negative bias was less than 10% for removal-depletion population estimates of Atlantic salmon *Salmo salar* parr where *N* was greater than 20, and true catchability was greater than 0.50 (and decreased 10% between electrofishing passes). Ultimately, electrofishing capture efficiency depends on the ability of the gear operators (Riley and Fausch 1992; Rosenberger and Dunham 2005; Sweka et al. 2006). Experienced electrofishing crews may catch more of the low-catchability fish, which can cause inherent efficiency variation within a population (Bohlin and Sundstrom 1977). Seven of our eight gear operators and netters had 10 or more years of removal-depletion sampling experience.

Removal-depletion capture efficiencies we obtain in typical southern Appalachian trout streams with low conductivity and limited habitat complexity are routinely greater than 50%, exceeding those reported in previous evaluations of three-pass depletion sampling (20–57%, Peterson et al. 2004; 44% first pass mean and lower thereafter, Rosenberger and Dunham 2005). Habitat notwithstanding, these capture efficiency differences could also be related to different levels of immobilization in the earlier studies, although they do not provide that information. Additionally, Rosenberger and Dunham's (2005) capture efficiencies were probably depressed somewhat because they combined all trout longer than 60 mm (fork length). We captured, on average, 88% of trout over 100 mm and 65% of trout under 100 mm in the experimental populations subject to three-pass depletion sampling in this study. Even the relatively unbiased first-pass capture efficiencies we obtained (74%, >100 mm; 46%, <100 mm) were typically higher than those reported by Peterson et al. (2004) and Rosenberger and Dunham (2005). Our capture efficiencies could be somewhat inflated because experimental fish did not have the 24–48 h postmarking and handling recovery time recommended by Peterson et al. (2004), which may alter behavior and physiology (Mesa and Schreck 1989). However, Temple and Pearsons (2006) found that rainbow trout catchability did not significantly differ between 24 h and 3 h recovery periods following electrofishing, marking, and release (shorter recovery periods were not evaluated).

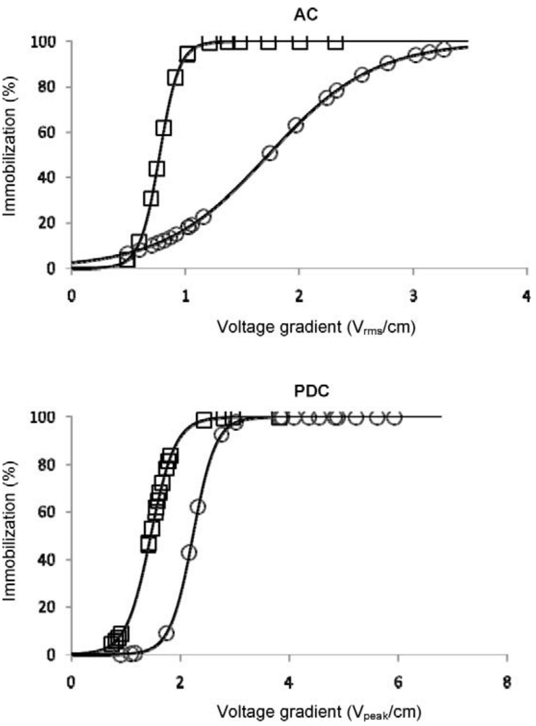

FIGURE 2.—Effectiveness of 60-Hz AC (upper panel) and pulsed DC (lower panel) for inducing immobilization within 5 s of exposure in rainbow trout less than 100 mm long (circles) and 100 mm or more (squares). The curves were generated by logistic regression of the binary response (immobilization or no immobilization within 5 s) for individual fish. The points on the curves indicate the particular voltage gradients to which individual fish were exposed.

Notwithstanding the relatively short recovery times in this study, trout capture efficiencies we have obtained during multiple-pass (electrofishing) rainbow trout eradication projects in other low-conductivity streams corroborate our results. Kulp and Moore (2000) captured 81% of trout greater than 100 mm and 70% of all trout they removed from an 858-m treatment reach with their first three-pass effort (one electrofishing unit). The initial three-pass effort in a larger Tennessee stream (mean width, 3.6 m; two electrofishing units) captured 88% of trout longer than 100 mm and 83% of the 2,066 rainbow trout present in a 1.9-km reach (our unpublished data). In that case, a second three-pass effort before the next spawning season was enough to prevent reproduction in 15 of 18 treatment reach sub-sections, which is unlikely if the true initial adult capture efficiency was markedly lower. Thompson and Rahel (1996) obtained similar removal efficiencies for a single three-pass electrofishing effort to eliminate brook trout *Salvelinus fontinalis* from three Rocky Mountain streams. Although brook trout remained the following year, density was greatly reduced and recruitment essentially halted. Meyer et al. (2006b) estimated brook trout capture efficiencies of 79–88% for a removal project in another Rocky Mountain stream, but that effort was unsuccessful because of undetected fish upstream of the treatment area, a questionable fish barrier downstream, and relatively complex habitat (K. Meyer, Idaho Department of Fish and Game, personal communication).

Our results provide further evidence that some fish in a given population have electrofishing capture probabilities that are effectively zero, even where habitat complexity is limited. Ninety-five trout under 100 mm remained at large long enough to experience one to three additional depletion efforts (three to nine additional passes), but 60% of those were never recaptured. The corresponding rate for trout above 100 mm was even higher (72% of 46 fish). The block nets used during the 2-d sampling period remained intact overnight; thus, there was a low likelihood of escapement. Previously, Bohlin and Sundstrom (1977) were unable to completely recapture experimental trout populations ($N = 150$) introduced into a natural stream after 20 electrofishing passes. Where habitat is more complex uncatchable fish can hinder efforts to remove an undesirable trout population (Habera et al. 1992) and increase negative bias associated with removal-model abundance estimates (Rosenberger and Dunham 2005).

Larger rainbow trout (>100 mm) were immobilized at lower power levels than were smaller trout (<100 mm, which was consistent with the higher capture efficiencies obtained elsewhere for depletion sampling (Lohr and West 1992; Thompson and Rahel 1996; Peterson et al. 2004; Korman et al. 2009). Consistency between the laboratory immobilization results and capture efficiencies from the field provide further evidence that small (i.e., age-0) rainbow trout are less vulnerable to capture by electrofishing than larger adult fish. They must encounter the higher voltage densities that occur closer to the electrodes to be immobilized.

### *Management Implications*

Size-specific measured capture efficiencies obtained in this study were within the range (50–100%) reported for three-pass depletion samples from other low-conductivity trout streams (Moore et al. 1983; Lohr and West 1992). They were also similar to those obtained in higher-conductivity trout streams (Thompson and Rahel 1996). Even though capture efficiencies were relatively high, our removal depletions still underestimated true population size. However, these underestimation errors were relatively small for trout over 100 mm (upper 95% confidence limits were often within 10% of $N$ and always within 20% of $N$). We maintain that this level of accuracy would be sufficient for typical inventory needs and would provide, at least, a reliable index of true abundance for monitoring purposes. Use of a removal estimator that accounts for variable catchability, such as the generalized model of Otis et al. (1978), can help reduce negative bias (Sweka et al. 2006).

We believe it is essential to stratify electrofishing data by fish size to help offset catchability variation (Armour et al. 1983). Although we have performed no detailed investigations to determine how many size-classes are appropriate, the two used in this study (with 100 mm as the boundary) have worked reasonably well. This generally defines the adult and age-0 segments of wild trout populations in southern Appalachian streams during the summer sampling season.

Both electric fields we studied performed adequately in our low-conductivity sample stream. Voltage and duty cycle settings for PDC electrofishing gear should be based upon effectively capturing the target species while minimizing injury. Our results with PDC gear were based on operators using a hand-held dip net along with the anode. We believe this is an important means for maximizing capture efficiency, which is ultimately determined by gear operator ability. We have observed that the operator has the best (and possibly only) opportunity to capture some fish, particularly trout less than 100 mm, given their small zone of complete immobilization (radius of about 40 cm from anode). Although both electric fields were equally functional, PDC gear might provide enough of a galvanotaxic effect to improve capture efficiency in streams with more complex habitat, such as undercut banks and woody debris accumulations.

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## References

Anderson, C. S. 1995. Measuring and correcting for size selection in electrofishing mark–recapture experiments. Transactions of the American Fisheries Society 124:663–676.

Armour, C. L., K. P. Burnham, and W. S. Platts. 1983. Field methods and statistical analyses for monitoring small salmonid streams. U.S. Fish and Wildlife Service Report FWS/OBS-83/33.

Bearlin, A. R., S. J. Nicol, and T. Glenane. 2008. Behavioral responses of Murray cod *Maccullochella peelii peelii* to pulse frequency and pulse width from electric fishing machines. Transactions of the American Fisheries Society 137:107–113.

Bohlin, T., S. Hamrin, T. G. Heggberget, G. Rasmussen, and S. J. Saltveit. 1989. Electrofishing: theory and practice with special emphasis on salmonids. Hydrobiologia 173:9–43.

Bohlin, T., and B. Sundstrom. 1977. Influence of unequal catchability on population estimates using the Lincoln index and the removal method applied to electrofishing. Oikos 28:123–129.

Cada, G. F., J. M. Loar, and M. J. Sale. 1987. Evidence of food limitation of rainbow and brown trout in southern Appalachian soft-water streams. Transactions of the American Fisheries Society 116:692–702.

Cross, D. G., and B. Stott. 1975. The effect of electric fishing on the subsequent capture of fish. Journal of Fish Biology 7:349–357.

Dauwalter, D. C., F. J. Rahel, and K. G. Gerow. 2009. Temporal variation in trout populations: implications for monitoring and trend detection. Transactions of the American Fisheries Society 138:38–51.

Dolan, C. R., and L. E. Miranda. 2003. Immobilization thresholds of electrofishing relative to fish size. Transactions of the American Fisheries Society 132:969–976.

Dolan, C. R., and L. E. Miranda. 2004. Injury and mortality of warmwater fishes immobilized by electrofishing. North American Journal of Fisheries Management 24:118–127.

Ensign, W. E., J. W. Habera, and R. J. Strange. 1991. Food resource competition in southern Appalachian brook and rainbow trout. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 43(1989):239–247.

Flebbe, P. A., and C. A. Dolloff. 1995. Trout use of woody debris and habitat in Appalachian wilderness streams of North Carolina. North American Journal of Fisheries Management 15:579–590.

Gatz, A. J. Jr., J. M. Loar, and G. F. Cada. 1986. Effects of repeated electroshocking on instantaneous growth of trout. North American Journal of Fisheries Management 6:176–182.

Habera, J. W., and R. J. Strange. 1993. Wild trout resources and management in the southern Appalachian mountains. Fisheries 18(1):6–13.

Habera, J. W., R. J. Strange, B. D. Carter, and S. E. Moore. 1996. Short-term mortality and injury of rainbow trout caused by three-pass AC electrofishing in a southern Appalachian stream. North American Journal of Fisheries Management 16:192–200.

Habera, J. W., R. J. Strange, and S. E. Moore. 1992. Stream morphology affects trout capture efficiency of an AC backpack electrofisher. Journal of the Tennessee Academy of Science 67:55–58.

Henry, T. B., J. M. Grizzle, and M. J. Maceina. 2003. Comparison of in-water voltage gradients produced by electrofishing boats. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 55(2001):138–145.

Hintze, J. 2001. NCSS and PASS. Number Cruncher Statistical Systems, Kaysville, Utah.

House, R. 1996. An evaluation of stream restoration structures in a coastal Oregon stream, 1981–1993. North American Journal of Fisheries Management 16:272–281.

Kocovsky, P. M., and R. F. Carline. 2006. Influence of landscape-scale factors in limiting brook trout populations in Pennsylvania streams. Transactions of the American Fisheries Society 135:76–88.

Kolz, A. L., and J. B. Reynolds. 1989. Determination of power threshold response curves. U.S. Fish and Wildlife Service Technical Report 22:15–24.

Korman, J., M. Yard, C. Walters, and L. G. Coggins. 2009. Effects of fish size, habitat, flow, and density on capture probabilities of age-0 rainbow trout estimated from electrofishing at discrete sites in a large river. Transactions of the American Fisheries Society 138:58–75.

Kulp, M. A., and S. E. Moore. 2000. Multiple electrofishing removals for eliminating rainbow trout in a small southern Appalachian stream. North American Journal of Fisheries Management 20:259–266.

Lennon, R. E., and P. S. Parker. 1957. Electric shocker developments on Southeastern trout waters. Transactions of the American Fisheries Society 85:234–240.

Lohr, S. C., and J. L. West. 1992. Microhabitat selection by brook and rainbow trout in a southern Appalachian stream. Transactions of the American Fisheries Society 121:729–736.

Mesa, M. G., and C. B. Schreck. 1989. Electrofishing mark–recapture and depletion methodologies evoke behavioral and physiological changes in cutthroat trout. Transactions of the American Fisheries Society 118:644–658.

Meyer, K. A., D. J. Schill, J. A. Lamanasky Jr., M. R. Campbell, and C. C. Kozfkay. 2006a. Status of Yellowstone cutthroat trout in Idaho. Transactions of the American Fisheries Society 135:1329–1347.

Meyer, K. A., J. A. Lamanasky Jr., and D. J. Schill. 2006b. Evaluation of an unsuccessful brook trout electrofishing removal project in a small Rocky Mountain stream. North American Journal of Fisheries Management 26:849–860.

Miranda, L. E., and C. R. Dolan. 2003. Test of a power transfer model for standardizing electrofishing. Transactions of the American Fisheries Society 132:1179–1185.

Miranda, L. E., and C. R. Dolan. 2004. Electrofishing power requirements in relation to duty cycle. North American Journal of Fisheries Management 24:55–62.

Moore, S. E., B. Ridley, and G. Larson. 1983. Standing crops of brook trout concurrent with removal of rainbow trout from selected streams in Great Smoky Mountains National Park. North American Journal of Fisheries Management 3:72–80.

Neves, R. J., and G. B. Pardue. 1983. Abundance and production of fishes in a small Appalachian stream. Transactions of the American Fisheries Society 112:21–26.

Otis, D. L., K. P. Burnham, D. R. Anderson, and G. C. White. 1978. Statistical inference from capture data on closed populations. Wildlife Monographs 62.

Paul, A. J., J. R. Post, and J. D. Stelfox. 2003. Can anglers influence the abundance of native and nonnative salmonids in a stream from the Canadian Rocky Mountains? North American Journal of Fisheries Management 23:109–119.

Peterson, J. T., R. F. Thurow, and J. W. Guzevich. 2004. An evaluation of multipass electrofishing for estimating the abundance of stream-dwelling salmonids. Transactions of the American Fisheries Society 113:462–475.

Peterson, N. P., and C. J. Cederholm. 1984. A comparison of the removal and mark–recapture methods of population estimation for juvenile coho salmon in a small stream. North American Journal of Fisheries Management 4:99–102.

Platts, W. S., W. F. Megahan, and G. W. Minshall. 1983. Methods for evaluating stream, riparian, and biotic conditions. U.S. Forest Service General Technical Report INT-138.

Platts, W. S., and R. L. Nelson. 1988. Fluctuations in trout populations and their implications for land-use evaluation. North American Journal of Fisheries Management 8:333–345.

Riley, S. C., and K. D. Fausch. 1992. Underestimation of trout population size by maximum likelihood removal estimates in small streams. North American Journal of Fisheries Management 12:768–776.

Rosenberger, A. E., and J. B. Dunham. 2005. Validation of abundance estimates for mark–recapture and removal techniques for rainbow trout captured by electrofishing in small streams. North American Journal of Fisheries Management 25:1395–1410.

Seehorn, M. E. 1968. An inexpensive backpack shocker for one-man use. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 21(1967):516–524.

Silsbee, D. G., and G. L. Larson. 1983. A comparison of streams in logged and unlogged areas of Great Smoky Mountains National Park. Hydrobiologia 102:99–111.

Simonson, T. D., and J. Lyons. 1995. Comparison of catch per effort and removal procedures for sampling stream fish assemblages. North American Journal of Fisheries Management 15:419–427.

Sweka, J. A., C. M. Legault, K. F. Beland, J. Trial, and M. J. Millard. 2006. Evaluation of removal sampling for basinwide assessment of Atlantic salmon. North American Journal of Fisheries Management 26:995–1002.

Temple, G. M., and T. N. Pearsons. 2006. Evaluation of the recovery period in mark–recapture population estimates of rainbow trout in small streams. North American Journal of Fisheries Management 26:941–948.

Thompson, P. D., and F. J. Rahel. 1996. Evaluation of depletion-removal electrofishing of brook trout in small Rocky Mountain streams. North American Journal of Fisheries Management 16:332–339.

Thurow, R. F., J. T. Peterson, and J. W. Guzevich. 2006. Utility and validation of day and night snorkel counts for estimating bull trout abundance in first- to third-order streams. North American Journal of Fisheries Management 26:217–232.

Van Deventer, J. S., and W. S. Platts. 1989. Microcomputer software system for generating population estimates from electrofishing data: user's guide for Microfish 3.0. U.S. Forest Service General Technical Report INT-254. Updated version available: microfish.org. (September 2006).

White, G. C., A. R. Anderson, K. P. Burnham, and D. L. Otis. 1982. Capture–recapture and removal methods for sampling closed populations. Los Alamos National Laboratory, Report LA-8787-NERP, Los Alamos, New Mexico.

Zoellick, B. W., D. B. Allen, and B. J. Flatter. 2005. A long-term comparison of redband trout distribution, density, and size structure in southwestern Idaho. North American Journal of Fisheries Management 25:1179–1190.

Zippin, C. 1958. The removal method of population estimation. Journal of Wildlife Management 22:82–90.