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TITLE:

Modeling the Size Spectrum for Macroinvertebrates and Fishes in Stream Ecosystems

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ecology, fish, invertebrates, size-structure, scaling relationship, body mass, density, individual variation, ataxic data, linear regression, length-mass relationship, depletion sampling

SUMMARY:
This is a protocol to model the size spectrum (scaling relationship between individual mass and population density) for combined fish and invertebrate data from wadable streams and rivers. Methods include: field techniques to collect quantitative fish and invertebrate samples; lab methods to standardize the field data; and statistical data analysis.

ABSTRACT:
The size spectrum is an inverse, allometric scaling relationship between average body mass (*M*) and the density (*D*) of individuals within an ecological community or food web. Importantly, the size spectrum assumes that individual size, rather than species' behavioral or life history characteristics, is the primary determinant of abundance within an ecosystem. Thus, unlike traditional allometric relationships that focus on species-level data (e.g., mean species' body size vs. population density), size spectra analyses are 'ataxic' – individual specimens are identified only by their size, without consideration of taxonomic identity. Size spectra models are efficient representations of traditional, complex food webs and can be used in descriptive as well as predictive contexts (e.g., predicting responses of large consumers to changes in basal resources). Empirical studies from diverse aquatic ecosystems have also reported moderate to high levels of similarity in size spectra slopes, suggesting that common processes may regulate the abundances of small and large organisms in very different settings. This is a protocol to model the community-level size spectrum in wadable streams. The protocol consists of three main steps. First, collect quantitative benthic fish and invertebrate samples that can be used to estimate local densities. Second, standardize the fish and invertebrate data by converting all individuals to ataxic units (i.e., individuals identified by size, irrespective of taxonomic identity), and summing individuals

within $\log_2$ size bins. Third, use linear regression to model the relationship between ataxic *M* and *D* estimates. Detailed instructions are provided herein to complete each of these steps, including custom software to facilitate *D* estimation and size spectra modeling.

INTRODUCTION:

Body size scaling relationships, such as the positive association between body mass and metabolic rate, are well-known at the individual organism level and are now being studied at higher levels of organization[1-3]. These allometric relationships are most often power-law functions of the form $Y = aM^b$, where *Y* is the variable of interest (e.g., metabolism, abundance, or home range size), *M* is the body mass of a single or average individual, *b* is a scaling coefficient, and *a* is a constant. For statistical convenience, *Y* and *M* data are often log-transformed prior to analysis then modeled with linear equations of the form $\log(Y) = \log(a) + b \log(M)$, where *b* and $\log(a)$ become the linear model slope and intercept, respectively.

The size spectrum is a type of allometric relationship that predicts density (*D*, the number of individuals per unit area) or biomass (*B*, the summed mass of individuals per unit area) as a function of *M* (See Section 4 for additional information on the use of 'normalized' *D* or *B* estimates.) Like other scaling relationships between *M* and *D* or between *M* and *B*, the size spectrum plays a central role in basic and applied ecology. At the population-level, biologists often interpret negative $D \propto M$ relationships as evidence of density-dependent survival or as models of ecosystem carrying capacity (i.e., the 'self-thinning rule')[4,5]. At the community-level, $B \propto M$ relationships can be used to study system-level effects of anthropogenic perturbations, such as size-selective fishing[6,7]. Allometric scaling of *D* and *B* with *M* are also central to recent efforts to unite population, community, and ecosystem ecology[2,8,9].

One particularly important characteristic of the size spectrum is the fact that it is entirely ataxic[9,10]. This point is easy to miss when comparing scatterplots of $D \propto M$ or $B \propto M$ data but the distinction between **taxic** and **ataxic** models is a critical one. In taxic models, a single *M* value is used to represent the average body mass of every individual of a given species or taxa[11]. In ataxic models, all individuals within a data set are partitioned among a series of body size intervals or *M* bins, regardless of their taxonomic identity[12]. The latter, ataxic approach is advantageous in aquatic ecosystems where many taxa exhibit indeterminate growth and experience one or more ontogenetic shifts in feeding behavior; in these instances, a single species-level *M* average will obscure the fact that a species can fill different functional roles throughout its life history[9,13,14].

Here, we present a complete protocol to quantify the size spectrum within wadable streams and rivers. The protocol begins with field sampling methods to collect the necessary fish and benthic macroinvertebrate data. Fish will be collected through a 'three-pass depletion' sampling process. Abundance will then be estimated from the depletion data with the Zippin method[15]. In depletion sampling, individual fishes within a closed study reach (i.e., individuals can neither enter nor leave the enclosed reach) are removed from the reach through three successive samples. Thus, the number of remaining fishes will be progressively depleted. From this depletion trend, total abundance within the study reach can be estimated then converted to *D* (in fish per m^2), using the known surface area of the study reach. Benthic macroinvertebrates will be collected with standard fixed-area samplers, then identified and measured in the laboratory.

Next, the combined fish and macroinvertebrate data will be partitioned among size bins. Traditionally, the octave or $\log_2$ scale (i.e., doubling intervals) has been used to set size bin boundaries[16]. Once a list of size bins has been established, partitioning of individual benthic macroinvertebrates among their respective size bins is straightforward because invertebrates are directly enumerated as numbers of individuals per unit area. However, estimating fish abundances within size bins is more abstract because these estimates are inferred from the depletion data. Detailed instructions are therefore provided to estimate fish abundance within size bins, irrespective of taxonomic identity, from depletion sample data.

Finally, linear regression will be used to model the size spectrum. This protocol is fully compatible with the original, general method of Kerr and Dickie[16] and identical to the methods used by McGarvey and Kirk, 2018[17] in a study of fish and invertebrate size spectra in West Virginia streams. By using this protocol, investigators can insure that their results are directly comparable with other studies that build upon Kerr and Dickie[16], thereby accelerating a broad and robust understanding of body size scaling relationships in freshwater ecosystems and the mechanisms that drive them.

PROTOCOL:

All methods described here have been approved by the Institutional Animal Care and Use Committee (IACUC) of Virginia Commonwealth University.

1. Collection and processing of fish samples

1.1 Isolating fishes within the study reach to create a closed fish assemblage

1.1.1 Identify the upstream and downstream (direction is relative to a surveyor facing 'upstream' and **against** the water current) ends of the study reach then mark the ends with removable flagging tape.

NOTE: The total length of the study reach is arbitrary but should be long enough to encompass a representative selection of the different habitat types (e.g., riffles, runs, pools, undercut banks) present within the entire stream. In most cases, a 100—200 m study reach will be adequate.

1.1.2 Measure the width of the wetted stream channel at 5—10 transects, distributed evenly along the length of the study reach. Estimate the total surface area of the study reach as the average wetted channel width multiplied by the total length of the reach.

1.1.3 Secure block nets (i.e., knotless seines with floats on the top line and weights on the bottom line) across the stream channel at the upstream and downstream ends of the study reach. Use medium to coarse mesh nets (6.3—9.5 mm or ¼—3/8 inch mesh) to minimize accumulation of debris and clogging. Do not use large (>12.7 mm) mesh nets as small fishes will pass through.

NOTE: Prior to sampling, prepare a rigging kit that includes: (i) 8 long (≥15 m length) pieces of polypropylene rope (9.5 mm or 3/8 inch diameter); and (ii) 8 **cam-action** tie-down straps. Do not use ratchet-action straps as these can snap and cause serious injury when their load is released.

1.1.3.1 At the upstream end of the study reach, locate a tree, root, large rock, or other solid object that can be used to anchor a net on each side of the stream. The availability of suitable anchor points on each side of the stream will likely affect the position of the upstream boundary.

1.1.3.2 Select one piece of polypropylene rope and create a loop at each end using a bowline knot. Use only a bowline knot, as other knots may become permanently sealed when exposed to moisture and high tension. For instructions on tying a bowline knot, see **Figure 1**.

1.1.3.3 Wrap the rope around the tree/root/rock and feed the loop at one end through the loop at the other end to create an anchor point (**Figure 2**). Shorten or lengthen the rope anchor by adding or removing wraps around the tree/root/rock.

1.1.3.4 Repeat steps 1.1.3.1—1.1.3.3 to establish a second anchor point on the opposite side of the stream.

1.1.3.5 Create a loop in the lines at each of the four corners of the block net using a bowline knot. Use only a bowline knot (**Figure 1**).

1.1.3.6 Connect both sides of the top line of the block net (the line with floats) to the anchor points using cam-action tie-down straps. Insert the hooks at either end of the tie-down strap into the loops at the corners of the block net and the anchor points (**Figure 2**). Pull the free tether of the tie-down strap through the cam buckle to tighten each point of contact.

NOTE: To release tension on the anchors (for adjusting the block net set-up or to remove the block net when sampling is complete), depress the cam button on each of the tie-down straps.

1.1.3.7 Secure the bottom line of the block net (the line with weights) by pinning them to the stream bank with tent stakes.

1.1.3.8 Establish a seal with the bottom of the stream using large rocks to pin the block net down. Place rocks on the side of the net facing upstream. Be sure that the top of the net remains above water level (**Figure 2**). Adjust the height(s) of the anchor point(s) as needed.

1.1.3.9 Set a second block net by repeating steps 1.1.3.1—1.1.3.8 at the downstream end of the study reach.

1.2 Perform the first of 3 fish sampling depletion passes within the enclosed study reach. This protocol assumes that a backpack electrofisher is available and all surveying crew personnel are properly trained to use it. Other methods can potentially be used but may not be as effective in collecting representative fish samples.

NOTE: In small streams, 4—5 people is an ideal crew size: one to operate the electrofisher, two to net stunned fishes, and one or two to carry holding buckets and shuttle captured fishes. Also, backpack electrofishing can cause significant injury, both to stream fishes and humans[18]. It is therefore critical to exercise caution and to receive proper training.

1.2.1 Beginning at the downstream end of the enclosed study reach, turn the backpack electrofisher on and move in the upstream direction. Progress slowly, moving side-to-side throughout the study reach to ensure all instream habitats are sampled. The first depletion pass is complete when the upstream net is reached.

1.2.2 Let supporting crew members follow the leader (who is operating the electrofisher), collecting stunned fishes with dip nets as they are spotted and transferring them to temporary buckets, then to aerated holding tubs. Use small battery powered 'bait bucket' pumps with aeration stones to ensure that captured fishes remain healthy.

1.2.2.1 Pay particular attention to very small, young-of-year fishes as they are difficult to spot and capture. When capture of the smallest fishes is highly inefficient, results may be biased. In this event, it may be necessary to remove the smallest $\log_2$ size classes from the fish data, prior to estimating densities within $\log_2$ size bins (see Step 3.2.2).

NOTE: Success in netting stunned fishes will vary with a number of biological and environmental conditions. For instance, turbid water in which visibility is low will constrain the ability to effectively locate and capture fishes; if turbidity is too high, sampling should be re-scheduled, or an alternate sampling site should be selected.

1.3 Processing fishes collected in the first depletion pass

1.3.1 Determine whether anesthesia will be needed. Live fishes are often difficult to handle, and sedation may be necessary to minimize stress and injury to fish specimens. If anesthesia is used, two options are widely (as of April 2019) available: Tricaine-S (tricaine methanesulfonate, MS-222) and carbon dioxide (baking soda).

NOTE: Tricaine-S entails a 21-day holding period before exposed fish can safely be consumed[19,20], but it is currently (as of April 2019) the only fish sedative approved by the U.S. Food and Drug Administration.

1.3.2 When using sedatives, carefully follow all instructions provided with the anesthetic product. In all cases, mix the anesthetic compound in an aerated water bath. Submerge collected fishes in the bath until sedation is observed. Once sedated, process the fishes as quickly as possible, as prolonged exposure to sedatives may cause death.

1.3.3 Use small dip nets to retrieve sampled fishes from the holding tank (with or without sedation), individually or in small batches, for identification. Place the specimens in white plastic or enamel trays and use forceps and magnifying glasses for examination. Use local or regional identification keys (e.g., “The Fishes of Ohio”)[21] to aid in identification.

1.3.4 Measure total length (from tip of snout to end of caudal fin) for each specimen then weigh on a field balance. If using an electronic balance, select one with 0.1 or 0.01 g precision. Keep a transparent plastic box on hand to use, as necessary, as a wind and rain baffle (it must be large enough to cover the balance and specimens being weighed).

1.3.5 Record all information (species identity, total length, and weight) on waterproof data sheets. A printable example of a fish data sheet is provided in **Supplementary File 1**.

1.3.6 Once processed, return the fishes to a separate aerated holding/recovery bin. When all fishes have been processed, release them **downstream** of the downstream block net.

NOTE: If you accidentally release them into your enclosed study reach, you will ruin your sample! If anesthesia was used, wait to release until all fishes have recovered and regained equilibrium.

1.4 Performing the second and third depletion passes

NOTE: If a strong depletion trend is not induced in the first three passes (i.e., if the number of sampled fishes has not noticeably declined by the third pass), additional passes may be needed to accurately estimate fish abundance[22]. Time permitting, it is often a good idea to proactively conduct four or five successive depletion passes.

1.4.1 Check that the upstream and downstream block nets are still secure. If significant debris has been collected in either block net, remove it by hand picking.

1.4.2 Collect the remaining depletion pass samples by repeating steps 1.2–1.3. Ensure that sampling effort remains consistent among all three passes. Use the same pace of movement (timing the process is recommended) and same crew members to resurvey the sampling reach.

1.5 When finished, disassemble the block nets and remove all anchor materials.

2. Collection and processing of benthic macroinvertebrate samples

2.1 Select benthic macroinvertebrate sample sites within the boundaries of the fish sampling reach that are representative of the major types of physical habitats (e.g., riffles or runs) observed in the study reach.

2.2 Using a fixed-area sampler, collect the first benthic macroinvertebrate sample. In shallow streams with extensive gravel-to-pebble size material, the Surber sampler and Hess sampler are the most commonly used devices but any fixed-area sampler can be used. When sampling other types of habitats where these devices do not work, consult Merritt *et al.*[23] and Hauer and Resh[24].

2.2.1 Place the sampling device firmly against the stream bottom with the sample collection net oriented downstream; move large cobbles as necessary to establish a firm seal with the substrate.

2.2.2 Use a wire or plastic brush to vigorously scrub the substrate within the sampling area for a period of 2 min, allowing dislodged benthic macroinvertebrates to drift into the sample net.

2.2.3 Transfer the sample contents from the net to a plastic jar and cover with 70% isopropyl alcohol for preservation. Label the jar and store it in a safe location for transfer to the lab.

2.3 Collect and preserve additional benthic macroinvertebrate samples, repeating step 2.2.

NOTE: The number of macroinvertebrate samples that should be collected is variable and somewhat arbitrary. Ideally, 5—10 replicate samples should be collected and individually preserved. At a minimum, 3 replicate samples should be collected.

2.4 Return all collected samples to the lab for processing.

NOTE: Isopropyl alcohol is a flammable liquid and if preserved samples will be shipped via ground or air carrier, it will be necessary to first complete and satisfy all pertinent hazardous goods/dangerous goods training, packing, and shipping requirements.

2.5 In the lab, sort and identify preserved benthic macroinvertebrate samples.

2.5.1 Separate thespecimens from fine sediment by carefully pouring sample contents into a fine-mesh sieve (e.g., 125 or 250 µm) and rinsing.

2.5.2 Transfer rinsed contents to a white plastic or enamel tray, cover with a small volume of water, and manually pick macroinvertebrates from the remaining residue with fine point forceps. Place extracted macroinvertebrates in a small container of 70% isopropyl alcohol.

NOTE: If a large amount of coarse plant or mineral residue is mixed with the sample contents, making it difficult to see macroinvertebrates in the tray, it may be necessary to process the remaining sample contents by first subdividing the material and working with several smaller quantities.

2.5.3 Using a dissecting stereo microscope with an ocular micrometer installed in one of the eyepieces, identify specimens to the lowest practical taxonomic level. In most instances, this will be family or genus level.

NOTE: Processing and identifying the complete contents of a single invertebrate sample will often require 2—5 h or longer. Budget sufficient time and be sure that a suitable library of taxonomic keys[25-28] is available to assist in identification.

2.5.4 Use the ocular micrometer in the microscope eyepiece to measure the complete body length of each specimen. If body length measurement is not possible (e.g., damaged or missing abdomen), measurement of the head capsule width may suffice.

2.5.5 Estimate individual dry mass (*M*) for each specimen using the body length or head capsule width measurements and taxon-specific body length vs. *M* or head width vs. *M* regression equations from published sources[29,30]. For example, the empirical body length (mm) vs. *M* (mg) equation reported in Benke *et al*.[29] for the alderfly *Sialis sp.* (Megaloptera, Sialidae) is $M = 0.0031 \times \text{total length}^{2.801}$. Therefore, estimated *M* for a *Sialis sp.* specimen with a total length of 15 mm is 6.104 mg.

NOTE: If a published length vs. *M* equation is not available for a particular taxon, substitute an appropriate equation at a higher level of taxonomic resolution (e.g., substituting the appropriate family level equation when the genus level equation is not available) or from a closely related taxon with a similar body shape.

3. Estimation of fish and benthic macroinvertebrate densities within $\log_2$ size bins

3.1 Establish a series of $\log_2$ size bins that will encompass all invertebrate and fish specimens, ranging from the smallest benthic macroinvertebrate to the largest fish. Ensure that all size estimates are in units of mg dry mass.

NOTE: For consistency, we recommend the size bins used by McGarvey and Kirk[17]. These size bins range from 0.0001 to 214,748.3648 mg. A spreadsheet with the lower and upper limits for each of these 31 $\log_2$ size bins is provided in **Supplementary File 2**.

3.2 Estimate fish abundance within each of the corresponding size bins.

3.2.1 First convert all individual fish weights from g wet mass (recorded on the field data sheets) to mg dry mass. The wet-to-dry mass conversion factor of Waters[31] (1 g wet mass = 0.2 g dry mass) can be used after converting from g to mg.

3.2.2 Sum the total number of individual fishes that were captured within each of the respective size bins (irrespective of species identity) during the first, second, and third depletion samples. An example is shown in **Supplementary File 2**.

NOTE: Biased under-sampling of very small fishes is common in steam fish sampling and will be evident when individuals are summed within $\log_2$ size bins; summed abundances of the smallest fishes will be conspicuously lower than in larger bins (e.g., 5 vs. 100 individuals in adjacent size bins). Remove $\log_2$ size bins that are clearly biased prior to size spectra analysis (see Step 4).

3.2.3 Use the Zippin maximum likelihood equation[15,32] to estimate total fish abundance (*n*) within the smallest fish size bin.

3.2.3.1 Begin by calculating an intermediate *X* statistic as

$$X = \sum_{i=1}^{k}(k-i)C_i \,, \qquad \text{(Equation 1)}$$

where *i* denotes the i^{th} sampling pass (*i* = 1, 2, 3, etc.), *k* represents the total number of passes (*k* = 3, unless additional passes were surveyed), and C_i is the total number of fishes captured during the i^{th} pass.

3.2.3.2 Calculate the maximum-likelihood estimate of *n* by iteratively substituting decreasing *n* values in Equation 2 until

$$\left[\frac{n+1}{n-T+1}\right]\prod_{i=1}^{k}\left[\frac{kn-X-T+1+(k-i)}{kn-X+2+(k-i)}\right]_i \leq 1.0, \qquad \text{(Equation 2)}$$

where *T* is the total number of individuals captured during *k* passes and all remaining variables are as defined above in Equation 1.

3.2.3.3 If zero counts are observed in the first, second, or third depletion sample, estimate *n* as the sum of individuals that were capture among the three depletion samples. A worked example of Equations 1 and 2 is shown in **Supplementary File 2**.

NOTE: Several software applications can be used to calculate Zippin abundance estimates from depletion samples, such as the 'removal' function in R package 'FSA' (Fisheries Stock Assessment)[33]. However, it is more instructive to manually solve Equations 1 and 2 in a spreadsheet. Detailed instructions are provided in Lockwood and Schneider[34] and **Supplementary File 2**.

3.2.4 Repeat step 3.2.3 for each of the remaining fish size bins.

3.2.5 Convert the *n* estimate for each size bin containing fish to a per 1 m^2 *D* estimate by dividing *n* by the total surface area estimate of the surveyed reach from step 1.1.2 above. For instance, if the Zippin *n* estimate is 70 fish and the surface area of the surveyed reach is 1,200 m^2, then *D* = 0.058 fish/m^2.

3.3 Estimate benthic macroinvertebrate abundance within $\log_2$ size bins by pooling results from each of the field samples (i.e., combine results from the replicate samples into a single list of individual specimens), then summing the total number of individuals within each size bin.

NOTE: If the length-mass equations used in step 2.5.5 produce individual weight estimates in units of mg dry mass, no additional unit conversion is necessary for benthic macroinvertebrates.

3.4 Estimate benthic macroinvertebrate *D* within each size bin as

$$D = \frac{\text{summed abundance}}{\text{no. samples} \times \text{surface area of sampling device}}. \qquad \text{(Equation 3)}$$

For example, if 6 benthic macroinvertebrate samples were collected with a standard Hess device (surface area = 0.086 m^2) and a total of 110 individuals were counted within a given size bin, the *D* estimate for that size bin is 213 individuals/m^2.

3.5 Combine *D* results for fishes and benthic macroinvertebrates into a single table of *D* estimates per $\log_2$ size bin. If fish and macroinvertebrates occur in the same size bin (a rare event that may occur for the largest invertebrates and smallest fishes), sum their respective *D* estimates to obtain a total *D* estimate for that size bin.

3.6 Delete any 'empty' $\log_2$ size bins (i.e., size bins with *D* values of zero), as empty bins will bias the linear regression models that are used to estimate size spectra parameters[35,36].

4. Modeling the benthic macroinvertebrate and fish size spectrum

4.1 Estimate the average dry mass for each $\log_2$ size bin ($\acute{M}$) using one of the following values: (i) the minimum value (lower boundary) for each size bin; (ii) the maximum value (upper boundary); (iii) the arithmetic mean (of the minimum and maximum); or (iv) the geometric mean (of the minimum and maximum)[35].

NOTE: In the examples shown below, $\acute{M}$ was estimated as the arithmetic mean of each $\log_2$ size bin (see **Supplementary File 2**).

4.2 'Normalize' the *D* estimate for each $\log_2$ size bin by dividing it by its respective width (i.e., difference between the upper and lower boundary)[16,35]. This will prevent the non-uniform $\log_2$ size intervals from creating bias in the linear regression models that are used to estimate size spectra parameters[35,37,38].

4.3 Log_{10} transform all $\acute{M}$ and $\underline{D}$ data to convert the curvilinear $D \propto M$ relationship to a linear relationship. Then use ordinary least squares regression with the $\log_{10}(\acute{M})$ and $\log_{10}(\underline{D})$ data to model the size spectrum as

$$\log_{10}(\underline{D}) = \log_{10}(a) + b \log_{10}(\acute{M}), \qquad \text{(Equation 4)}$$

where $\log_{10}(a)$ is the intercept and *b* is the slope of the linear size spectrum model.

REPRESENTATIVE RESULTS:

Exemplar results, including original field data, are presented for Slaunch Fork, West Virginia, a small stream in southern West Virginia. Additional size spectra model results are also presented for two other streams in the same region: Camp Creek and Cabin Creek, West Virginia. These are the three study sites included in McGarvey and Kirk[17], but data presented here are from new samples collected in May 2015. A fully worked, manual example of the size spectra modeling process is included for the Slaunch Fork data in **Supplementary File 2**. Alternatively, all calculations can be automated with a custom size spectra application (see **Figure 3**) at http://bit.ly/SizeSpectra.

In each of the three study streams a clear, negative $\underset{\sim}{D} \propto \acute{M}$ relationship was detected for combined benthic macroinvertebrate and fish data (**Figure 4**). Size spectra slopes were all between -1.7 and -1.8, with overlapping 95% confidence intervals (i.e., ± 1.96 standard errors). This similarity in the size spectra slopes indicates that abundance decreases with increasing body size at approximately equal rates in all three streams. However, the differing size spectra intercepts show that differences in overall $\underset{\sim}{D}$ are variable among streams, with highest densities in Camp Creek (intercept = 0.71) and much lower densities in Cabin Creek (intercept = 0.07).

FIGURE AND TABLE LEGENDS:

Figure 1. Four-step illustration of the bowline knot. Original illustration was created by Luis Dantas and is available at https://commons.wikimedia.org/wiki/File:Bowline_in_four_steps.png. This image is freely distributed under a CC-BY-SA-3.0 Creative Commons license (http://creativecommons.org/licenses/by-sa/3.0/).

Figure 2. Illustration of block net set-up. Upper panel shows the general appearance and orientation of a secure block net. Lower panel emphasizes key steps to secure a block net.

Figure 3. Screen capture of the size spectra application. The software is hosted online (http://bit.ly/SizeSpectra) and all functions are accessed through a simple, graphical user interface.

Figure 4. Ataxic size spectra plots from three West Virginia streams. Benthic macroinvertebrate and fish data are distinguished by color. In each plot, average individual dry mass ($\acute{M}$) within $\log_2$ size bins is shown on the *x*-axis and normalized density ($\underset{\sim}{D}$) is shown on the *y*-axis. Least-squares regression lines are superimposed on each plot with linear model slopes (slo.), intercepts (int.), and coefficients of determination (r^2). Standard errors are included in parentheses for slopes and intercepts. To aid in comparison, all plot axes are shown at identical scales.

Figure 5. Taxic body mass vs. density relationship in Slaunch Fork. Each data point (diamond) represents the mean body mass (*M*, dry mass) and estimated density (*D*) of a single taxon. Linear regression models are shown separately for invertebrates and for fishes (dashed black lines), as well as combined taxa (solid gray line).

Supplementary File 1. Example field data sheet used to record fish identities, lengths, and weights.

Supplementary File 2. A fully worked example of the size spectra modeling process, using benthic macroinvertebrate and fish data (May 2015) from Slaunch Fork, West Virginia.

DISCUSSION:

This ataxic size spectra protocol can be used to quantify and model size structure within communities of stream fishes and invertebrates. Previous size spectra studies in stream ecosystems have ranged from basic descriptive research[39,40] to comparisons along a longitudinal river profile[41] and among distinct biogeographic regions[42]. Seasonal comparisons have been performed[43,44] and recently, seasonal changes in size spectra parameters have been linked to water temperature and hydrology[17]. Size spectra slopes have also been used to estimate trophic transfer efficiency among successive trophic levels[45,46], while size spectra intercepts have been used as proxies for food web capacity or ecosystem productivity[47,48]. These diverse examples demonstrate that size spectra models can be applied in many different contexts. Furthermore, when the necessary adjustments are made to the sampling methods, size spectra analyses are applicable to other types of ecosystems, including large rivers[48-50], lakes[51-53], and marine environments[54-56].

One question that may arise when considering a size spectrum analysis is whether ataxic size spectra models are fundamentally different than traditional $D \propto M$ models that use taxic data (i.e., a single average body mass and density estimate for each taxa)[57]. After all, taxic and ataxic models are both characterized by negative $D \propto M$ relationships that may appear similar when plotted on log-log axes. In principle, ataxic methods should be superior to taxic methods when the research objective is to understand how biomass is distributed or how energy is transferred in stream ecosystems[9]. This is because mean body mass estimates (for a given taxon) can obscure significant variation in individual size. Throughout their life histories, many aquatic organisms increase their body mass by several orders of magnitude and experience one or more ontogenetic shifts in feeding behavior[14,58,59]. The average body mass estimates used in taxic analyses may therefore be misleading, while ataxic methods allow the full range of observed body sizes to be retained in studies of body size scaling[16].

Practical differences between ataxic and taxic methods can also be demonstrated empirically. In **Figure 5**, we show the taxic $D \propto M$ relationship from Slaunch Fork, West Virginia, using the same benthic invertebrate and fish data that were used in the ataxic size spectrum plot in **Figure 4** (raw data for Slaunch Fork are included in **Supplementary File 2**). When M is estimated as the mean dry mass of all individuals and D is estimated as the sum of individuals (standardized to individuals per m^2) of a given taxon, the slope of the $D \propto M$ model (solid gray regression line in **Figure 5**) increases to -0.59. Furthermore, the negative $D \propto M$ relationship becomes a function of differences in M and D among major taxa groups (invertebrates vs. fishes); evidence of a significant $D \propto M$ relationship is weaker when invertebrates and fishes are examined separately (dashed regression lines in **Figure 5**). This is a stark contrast with the ataxic $\underline{D} \propto \acute{M}$ model, which reveals a smooth, nearly constant decrease in density as body mass increases (see **Figure 4**).

A key point of concern in size spectra analysis is the formatting of the ataxic $\acute{M}$ and $\underline{D}$ data. Three sequential steps – partitioning individuals among $\log_2$ size bins, normalizing the density estimate for each size bin, and $\log_{10}$ transformation of all $\acute{M}$ and $\underline{D}$ data (as detailed above) – should be completed before standardized size spectra models are compared[16]. But in many cases, studies that report size spectra results have utilized different methods[38]. For instance, some authors have used $\log_2$ size bins and log-transformed data but did not normalize their D estimates[39,42].

Others have partitioned their ataxic data among $\log_5$ or $\log_{10}$ size bins, with or without normalizing their *D* estimates[38,40,41]. On the 'Example Data & Result' page of the size spectra program (http://bit.ly/SizeSpectra), we include toggles to illustrate the effects that $\log_{10}$ transformation and normalization of the density estimates have on the observed $D \propto M$ relationship (see **Figure 3**). These visualizations demonstrate why it is important to follow the complete, sequential method presented in Kerr and Dickie[16] and detailed herein, particularly when comparisons will be made between different size spectra models.

Size spectra results can also be sensitive to the binning process that is used to partition individual specimens among size bins. For this reason, Edwards *et al*.[60] developed a maximum likelihood method to model the size spectrum that uses cumulative distributions of individual size, rather than binned size data. This new approach ensures that comparisons of size spectra parameters will not be biased by variable binning schemes. It is therefore an important advance in size spectra research. However, cumulative distributions of individual specimens cannot be used when a secondary method, such as the Zippin depletion estimation method used here or a comparable mark-recapture tagging method, is needed to estimate *D* for populations of interest; cumulative distributions will only work when *D* estimates can be inferred directly from raw sample contents. In the present context, cumulative distributions could be built for the benthic invertebrate data (counts per unit sample area), but not for the fish data (total abundance inferred from depletion samples). We therefore encourage others to use the specific size bins listed in **Supplementary File 2**. These size bins should work well for most stream studies (i.e., encompass the size range of most macrofauna that will be encountered in small streams) and if used consistently, will help to ensure that size spectra models from different systems are directly comparable.

Finally, we caution that the field sampling methods detailed here for benthic macroinvertebrates and fishes may underestimate *D* within some $\log_2$ size bins if other types of aquatic macrofauna are locally present. In temperate, wadeable streams and rivers, these other macrofauna will often consistent of crayfishes[61] and salamanders[62]. When feasible, additional steps may be taken to collect representative samples of these organisms. However, accurate estimates of crayfish and salamander densities can be difficult to obtain. For example, backpack electrofishers, seine nets, baited traps, and custom-built quadrat samplers have all been used to study crayfish density and size structure, but no one method is widely recognized as superior[63-65]. Appropriate steps to incorporate crayfishes and salamanders will therefore depend upon local environmental conditions and prior knowledge of the local biota. At a minimum, investigators should recognize that if crayfishes and/or salamanders are present but not sampled, the *D* estimates within larger $\log_2$ size bins will underestimate the true *D* values, as individual crayfish and salamander body masses are generally comparable to stream fish body masses.

Despite these concerns, a growing body of research on the aquatic size spectrum suggests that aquatic organisms may adhere to relatively simple *M* scaling laws, as predicted by Peters[1], Sheldon et al.[66], Andersen and Beyer[67], and others. Methods presented here can, if broadly adopted, help to build a large and geographically extensive database on size spectra in stream ecosystems. This will in turn facilitate critical understanding of the baseline dynamics that underlie the size spectrum and aid in applied efforts to anticipate how perturbations will affect size-structured stream communities.

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Figure 1

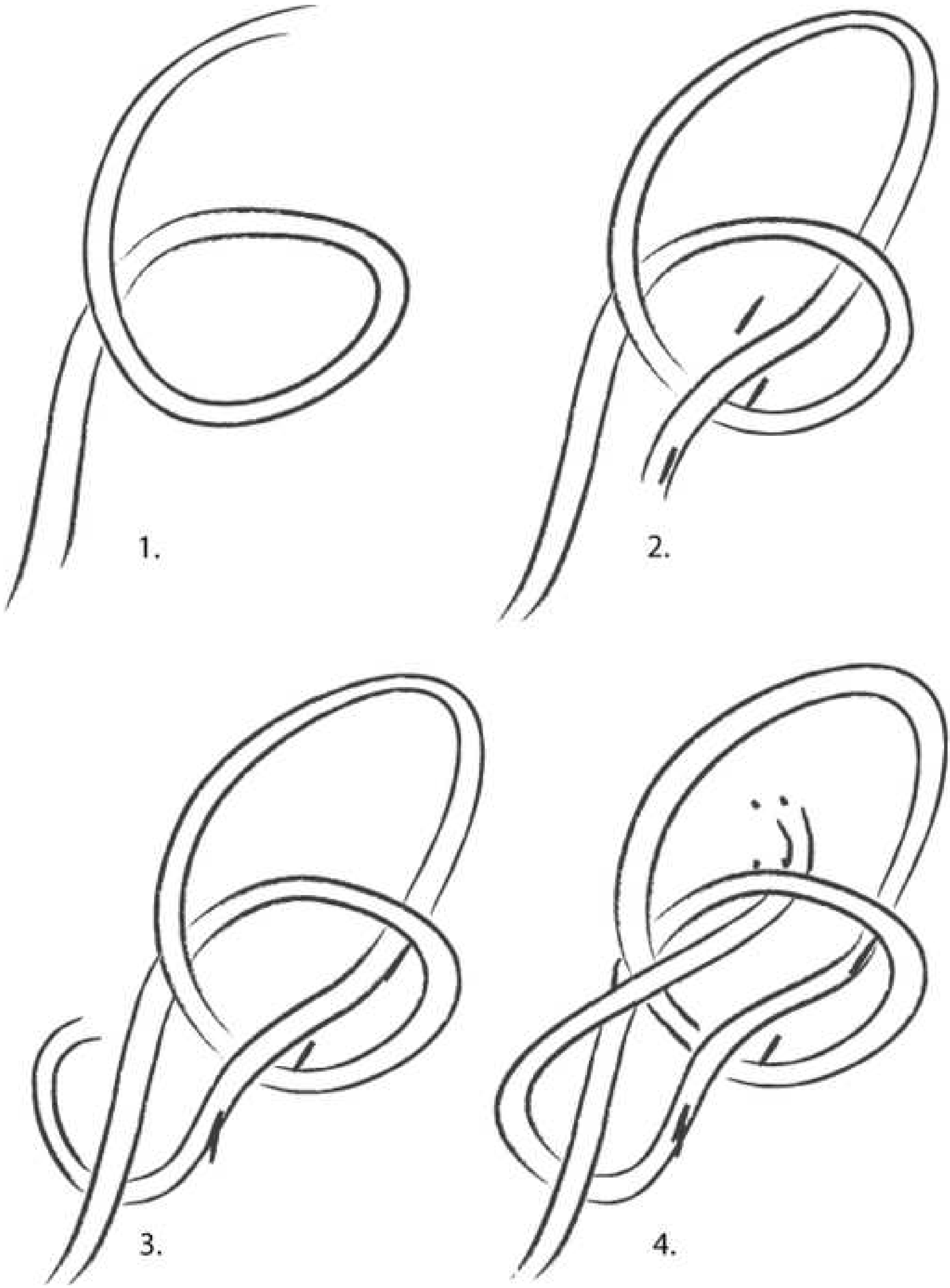

Figure 2

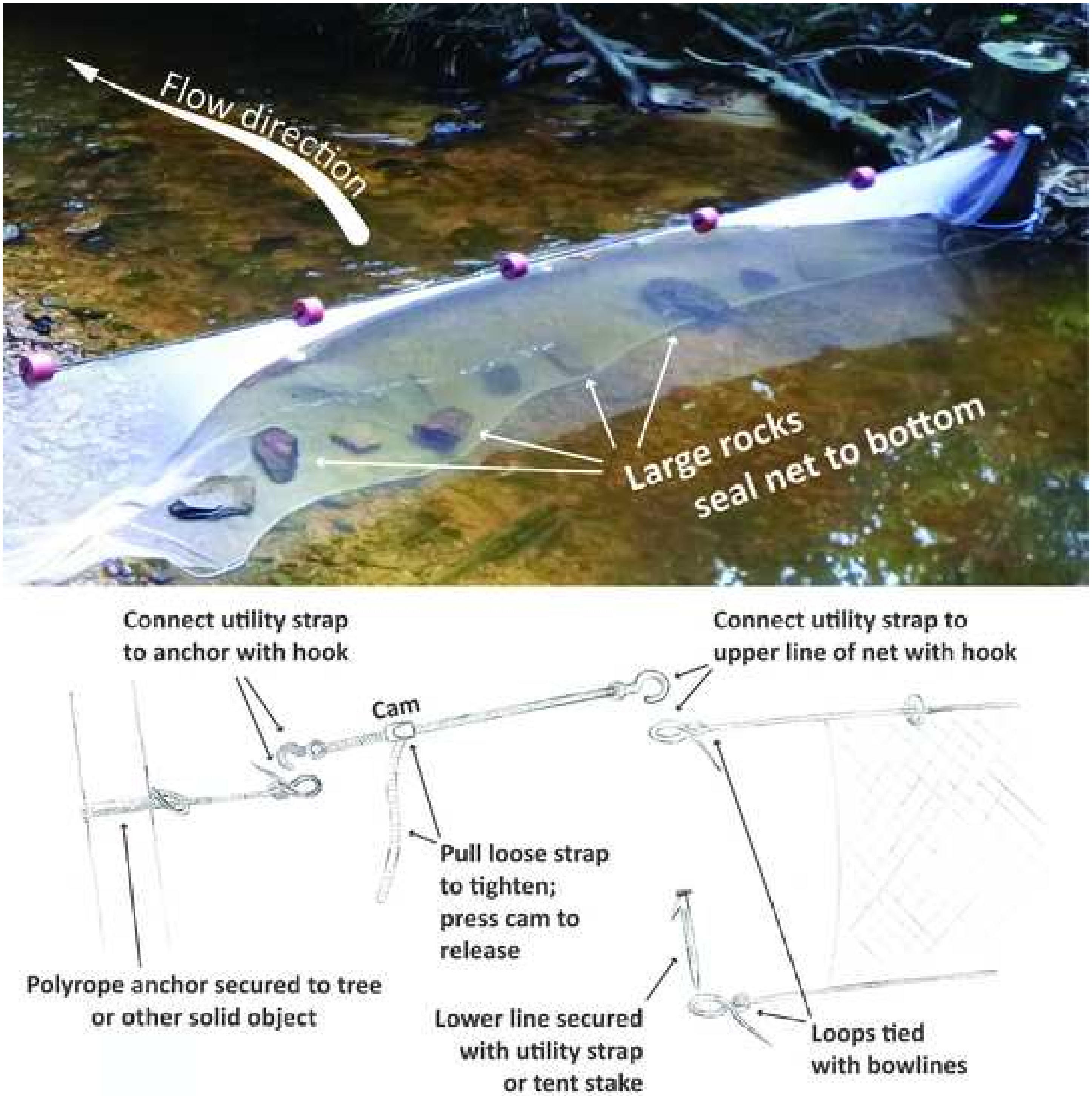
Flow direction
Large rocks
seal net to bottom
Connect utility strap
to anchor with hook
Cam
Connect utility strap to
upper line of net with hook
Pull loose strap
to tighten;
press cam to
release
Polyrope anchor secured to tree
or other solid object
Lower line secured
with utility strap
or tent stake
Loops tied
with bowlines

Figure 3

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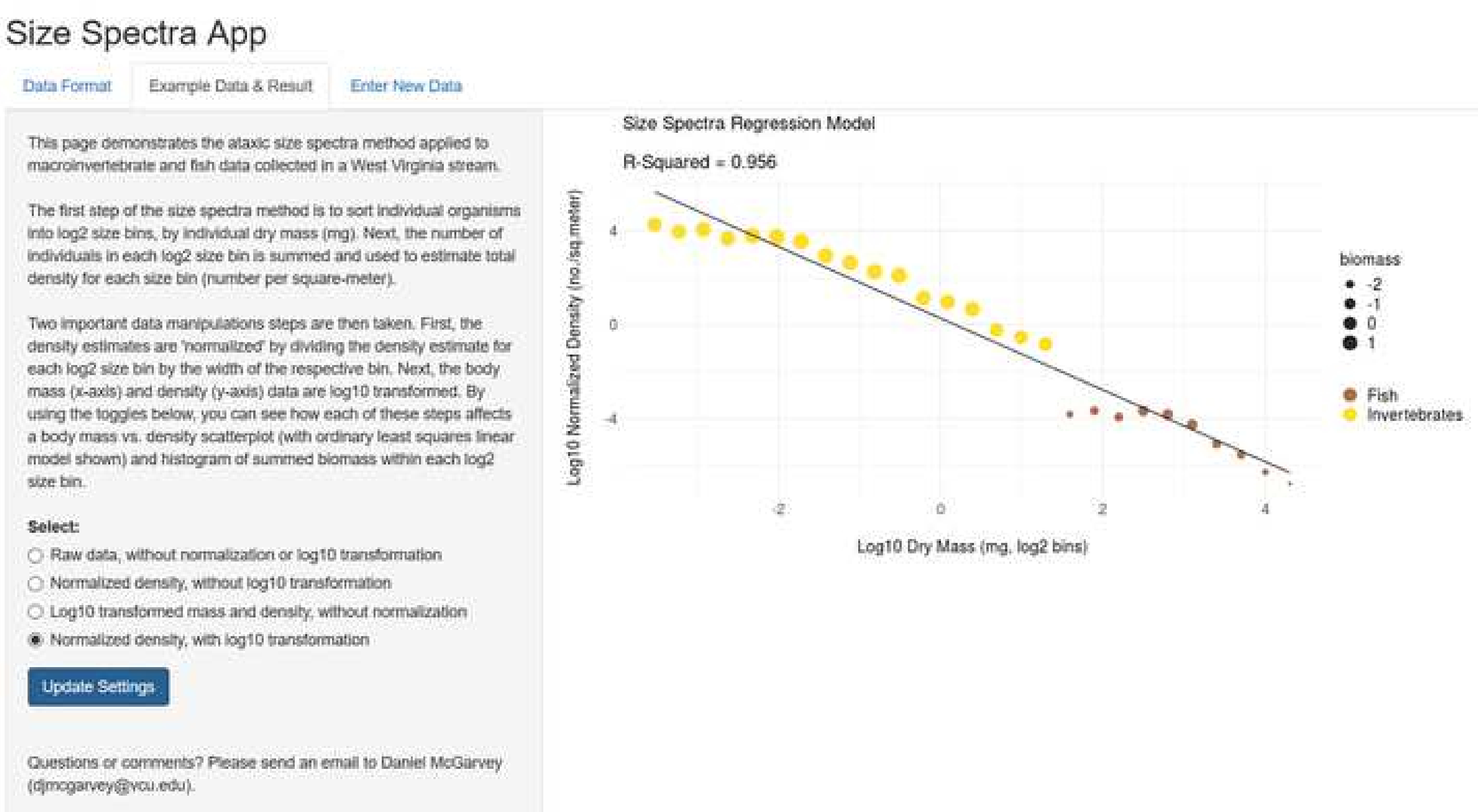

Figure 4

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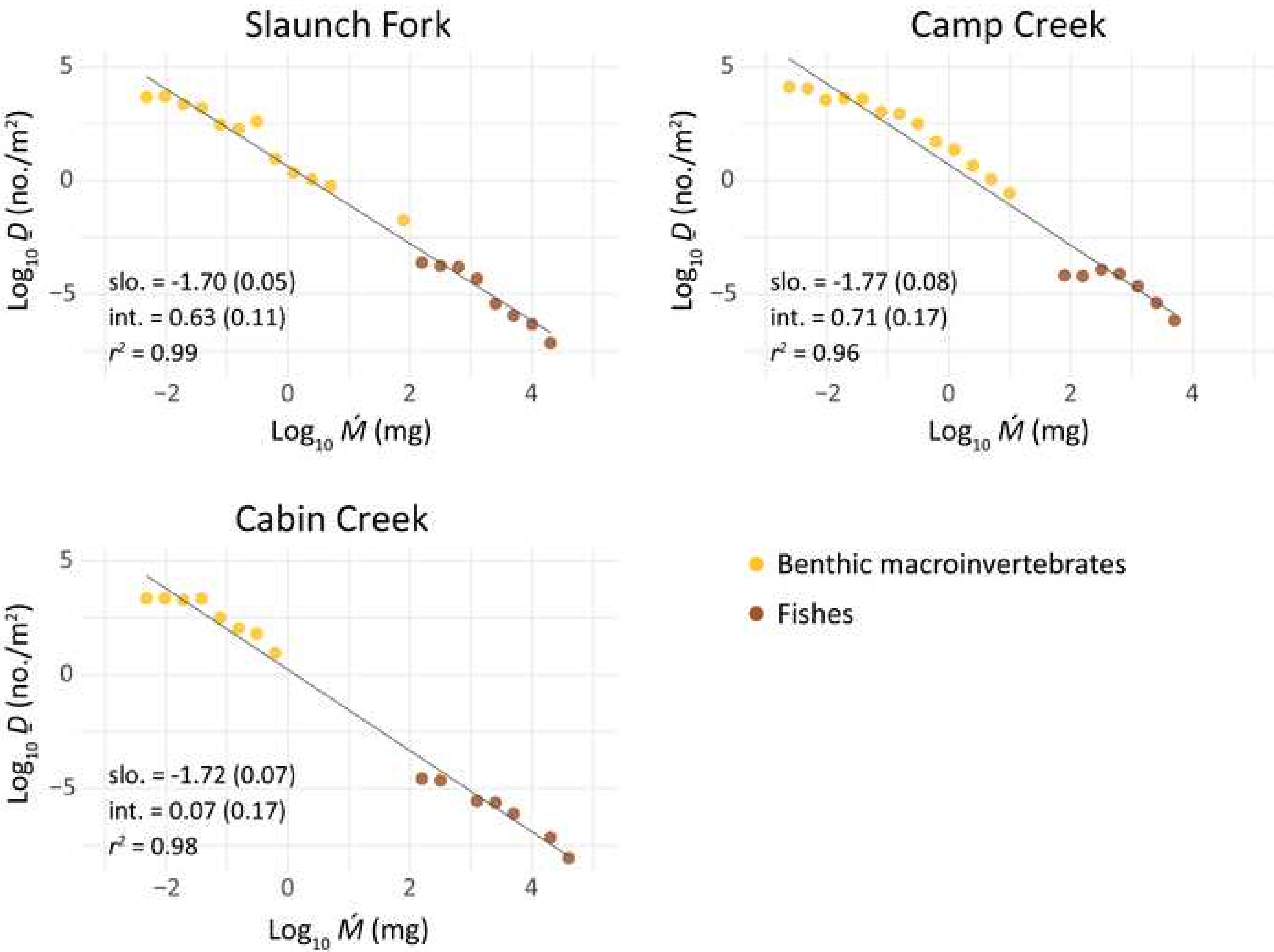

Figure 5

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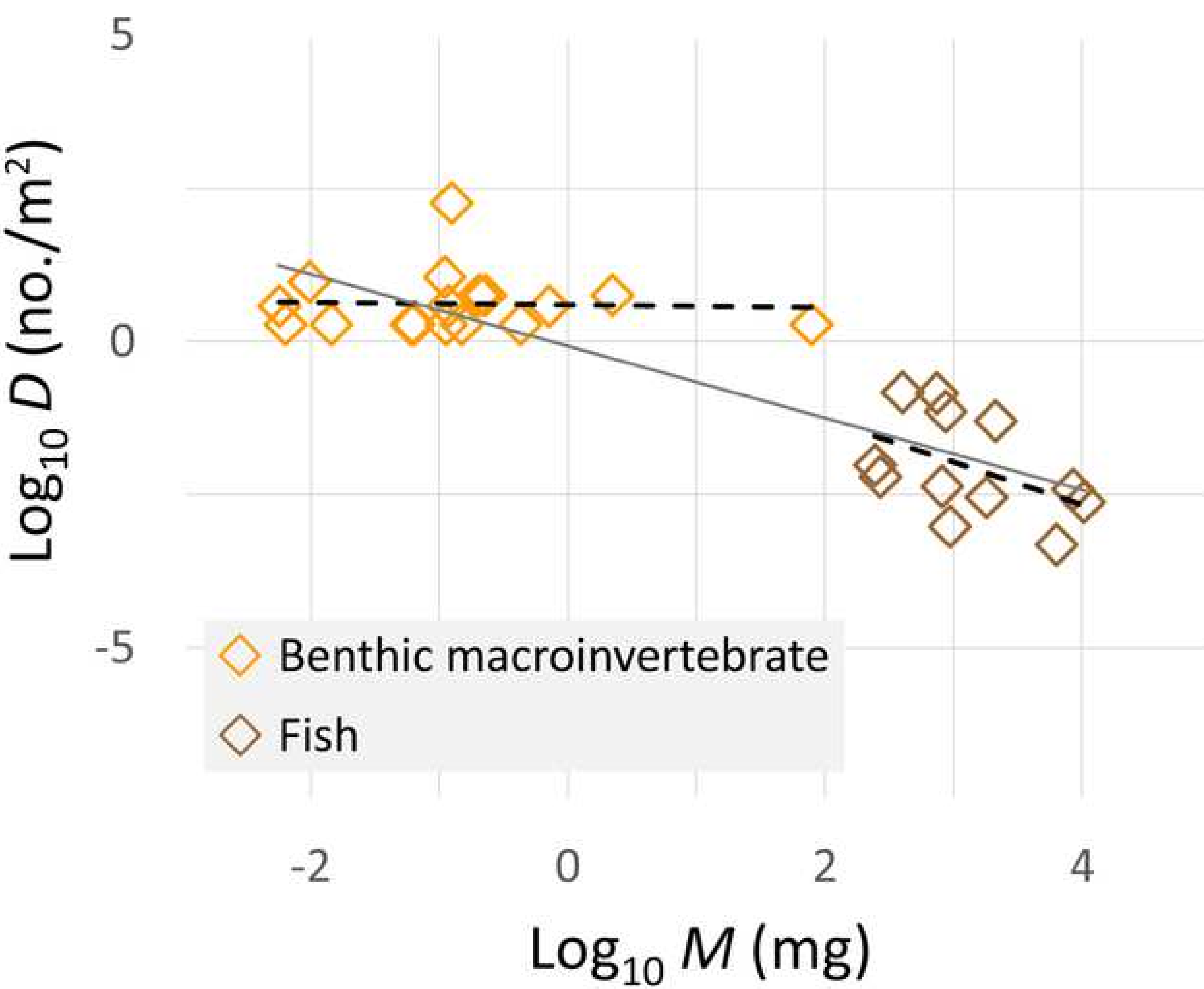

Name of Material / Equipment	Company	Catalog Number
Chest waders	Multiple options	n/a
Rubber lineman's gloves	Multiple options	n/a
Dip nets with fiberglass poles	Multiple options	n/a
Backpack electrofishing unit	Smith-Root; Halltech; Midwest Lake Management; Aqua Shock Solutions	www.smith-root.com; www.halltechaquatic.com; https://midwestlake.com; https://aquashocksolutions.com/
Block nets/seines (×2)	Duluth Nets	https://duluthfishnets.com/
Cam-action utility straps with 1 inch nylon webbing (×4)	Multiple options	n/a
Large tent stakes (×4)	Multiple options	n/a
5 gallon plastic buckets (×5)	Multiple options	n/a
10-20 gallon totes (×3)	Multiple options	n/a
Battery powered 'bait bucket' aeration pumps	Cabelas	IK-019008
Fish anesthesia (Tricaine-S)	Syndel	www.syndel.com
Folding camp table and chairs	Cabelas	IK-518976; IK-552777
Pop-up canopy	Multiple options	n/a
Fish measuring board	Wildco	3-118-E40
Battery powered field scale with weighing dish	Multiple options	n/a
Clear plastic wind/rain baffle	Multiple options	n/a
White plastic or enamel examination trays	Multiple options	n/a
Stainless steel forceps	Multiple options	n/a
Hand magnifiers	Multiple options	n/a
Fish identification keys	n/a	n/a
Datasheets printed on waterproof paper	Rite in the Rain	n/a
Retractable fiberglass field tapes	Lufkin	n/a
Surber sampler or Hess sampler	Wildco	3-12-D56; 3-16-C52
70% ethanol or isopropyl alcohol	Multiple options	n/a
Widemouth invertebrate specimen jars (20-32 oz.)	U.S. Plastic Corp.	67712

Comments/Description

Personal protective equipment for use during electrofishing. Do NOT use 'breatheable' waders as electrical current will pass through them.
Personal protective equipment for use during electrofishing.
Used to capture stunned fishes during electrofishing.

Backpack electrofishers are currently manufactured and distributed by four independent companies in North America. Prices and warranty,

Necessary length will depend on stream width. 3/8 inch mesh is recommended.
Used to secure/anchor block nets. Available at auto supply, hardware, and department stores.
Used to secure/anchor block nets. Available at camping and department stores.
Used to hold and transport fish during electrofishing. Available at hardware and paint supply stores.
Used as livewells, sedation tanks, and recovery bins for captured fishes. Available at hardware and department stores.
Used to aerate fish holding bins during field processing.
Used to sedate fishes for field processing. Tricaine-S is regulated by the U.S. Food and Drug Administration.
Used to process fish samples.
Used as necessary for sun and rain protection.
Used to measure fish lengths.
Used to weigh fishes. Must weigh be accurate to 0.1 or 0.01 grams.
Used to shield scale in rainy or windy conditions. Must be large enough to cover the scale and a weighing dish.
Trays are essential for examining fishes in the field.
Forceps are helpful when examining small fishes and in transfering invertebrates to specimen jars.
Magnification is often helpful when identifying fish specimens in the field.
Laminated keys that are custom prepared for specific locations are most effective.
Waterproof paper is essential when working with aquatic specimens.
Used to measure stream channel dimensions.
Either of these fixed-area benthic samplers will work well in shallow streams with gravel or pebble substrate.
Used as invertebrate preservative.
Any widemouth plastic jars will work but these particular jars are durable and inexpensive.

/technical support are the most important factors in choosing a vendor.

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Author Replies to Editor Comments

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1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

A final proofread has been completed and the authors do not believe that any spelling or grammar errors are present in the revised manuscript.

2. Please avoid long steps/notes (more than 4 lines).

All Methods steps and notes are now 4 lines or less in length. In several places, a new sub-step was added to create space for essential details.

3. The protocol should only contain numbered steps with few notes. Please remove other text (lines 95-101, 343-350, 443-447) to Introduction/Discussion.

The longer blocks of introductory/context text at the beginning of each main Methods sections have been moved to the Introduction, Discussion, or broken up and integrated with the Methods.

4. The highlighted protocol steps are over the 2.75 page limit (including headings and spacing). Please highlight fewer steps for filming.

The highlighted protocol is now at exactly 2 pages of single-spaced text, when blank spaces are not counted. To verify this, I created a "clean" copy of the revised manuscript and deleted all non-highlighted text. This copy has been uploaded to the submission system as "59945_R1_YellowHighlightOnly".

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All time units were converted as specified.

7. Step 3.2.3.3: Please write this step in the imperative tense.

Change was made to imperative tense.

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Stream Survey - Electrofishing Runs

Site: ______________________ Date: ______________

Electrofishing run# (1,2,3, etc.): ______ Start time: ________ End time: ________

Datasheet page: ______ of ______ Shock time (seconds): ________

Species	Total length (mm)	Wt (g)	Species	Total length (mm)	Wt (g)

Appendix 2. A fully worked example of the size spectra modeling process, using bentl

The example is distributed across the seven additional worksheets (tabs) included in this Excel file, an

Sheet 1. Log2_SizeBins

These are the 31 $\log_2$ size bins used in the stream size spectra analyses.

Benthic invertebrates and fishes are partitioned among these size bins, based upon their individual si

All units are milligrams (mg) dry mass.

Sheet 2. InvertebrateData_Raw

This is a list of combined benthic invertebrates, pooled across six Hess samples.

The dry mass of each individual was estimated using body length or head width measurements and p

Note: The invertebrate data on this sheet can be saved as a separate .csv file and uploaded to the onli

Sheet 3. InvertebrateData_Binned

These are tallied counts of benthic invertebrates (from Sheet 2) that fall within each of the correspon

The summed counts are then converted to per-square-meter densities using the surface area of the H

Sheet 4. FishData_Raw

These are results from each of the three consecutive fish depletion samples.

Individual specimens are identified by the run (Run 1, 2, or 3) that each was captured during, species'

Note: The fish data on this sheet can be saved as a separate .csv file and uploaded to the online Size Sp

Sheet 5. FishData_ZippinExample

This worksheet shows how population size can be estimated using the Zippin depletion method.

In this particular example, the numbers of fishes weighing between 838.86 - 1677.72 mg dry mass are

Note: Because the size spectra method is ATAXIC (i.e., only the size of each individual, rather than it's t

Sheet 6. FishData_Binned

These are estimated abundances of fishes (from Sheet 4) that fall within each of the corresponding lo

The abundance estimates are converted to per-square-meter densities using the known surface area

Sheet 7. CombinedData

These are the combined and fully formatted benthic invertebrate and fish data for size spectra analys

Separate columns are shown for normalized density and for $\log_{10}$ transformed dry mass and normaliz

hic macroinvertebrate and fish data (May 2015) from Slaunch Fork, West Virginia.
ıd is explained in sequence below.

ze.

ublished length-dry mass regression models.
ne Size Spectra Analysis program (http://bit.ly/SizeSpectra), after deleting the first four rows (i.e., leavi

ıding $\log_2$ size bins.
Iess sampler and the total number of Hess samples.

identity, and individual dry mass.
pectra Analysis program (http://bit.ly/SizeSpectra), after deleting the first four rows (i.e., leaving just th

e tallied for depetion Runs 1, 2, and 3.
taxonomic identity, is considered), fishes are partitioned among size bins IRRESPECTIVE of taxonomic id

ıg$_2$ size bins.
ı of the enclosed (with block nets) sampling reach.

sis.
ted density data.

ng just the column headers above the data).

ne column headers above the data).

lentity.

These are the log_2 size bins used in stream community size spectra analysis.

Potential estimates of average size bin dry mass include the lower and upper boundaries c

All units are milligrams (mg) dry mass.

Log_2 Size Bin Lower Boundary (mg dry mass)	Log2 Size Bin Upper Boundary (mg dry mass)
0.0001	0.0002
0.0002	0.0004
0.0004	0.0008
0.0008	0.0016
0.0016	0.0032
0.0032	0.0064
0.0064	0.0128
0.0128	0.0256
0.0256	0.0512
0.0512	0.1024
0.1024	0.2048
0.2048	0.4096
0.4096	0.8192
0.8192	1.6384
1.6384	3.2768
3.2768	6.5536
6.5536	13.1072
13.1072	26.2144
26.2144	52.4288
52.4288	104.8576
104.8576	209.7152
209.7152	419.4304
419.4304	838.8608
838.8608	1677.7216
1677.7216	3355.4432
3355.4432	6710.8864
6710.8864	13421.7728
13421.7728	26843.5456
26843.5456	53687.0912
53687.0912	107374.1824
107374.1824	214748.3648

of each log $_2$ size bin, as well as the arithmetic mean and ge

Log2 Size Bin Center as Arithmetic Mean (mg dry mass)
0.0002
0.0003
0.0006
0.0012
0.0024
0.0048
0.0096
0.0192
0.0384
0.0768
0.1536
0.3072
0.6144
1.2288
2.4576
4.9152
9.8304
19.6608
39.3216
78.6432
157.2864
314.5728
629.1456
1258.2912
2516.5824
5033.1648
10066.3296
20132.6592
40265.3184
80530.6368
161061.2736

ometric mean of each size bin.

Log2 Size Bin Center as Geometric Mean (mg dry mass)	Log2 Size Bin Bin Width (mg dry mass)
0.0001	0.0001
0.0003	0.0002
0.0006	0.0004
0.0011	0.0008
0.0023	0.0016
0.0045	0.0032
0.009	0.0064
0.0181	0.0128
0.0362	0.0256
0.0724	0.0512
0.1448	0.1024
0.2896	0.2048
0.5793	0.4096
1.1585	0.8192
2.317	1.6384
4.6341	3.2768
9.2682	6.5536
18.5364	13.1072
37.0728	52.4288
74.1455	104.8576
148.291	209.7152
296.5821	419.4304
593.1642	838.8608
1186.3283	1677.7216
2372.6566	3355.4432
4745.3133	6710.8864
9490.6266	13421.7728
18981.2531	26843.5456
37962.5062	53687.0912
75925.0125	107374.1824
151850.025	214748.3648

These are the raw benthic invertebrate data from Slaunch Fork, West Virginia (May 2015 samples)

Individuals in the table below have been pooled from six Hess samples and are shown with their respe

ORDER	FAMILY	GENUS	DRY_MASS_MG
Diptera	Chironomidae	na	0.0041
Diptera	Chironomidae	na	0.0041
Diptera	Chironomidae	na	0.0041
Diptera	Ceratopogonidae	Mallochohelea	0.0057
Diptera	Ceratopogonidae	Mallochohelea	0.0057
Coleoptera	Elmidae	Optioservus	0.0057
Coleoptera	Elmidae	Optioservus	0.0057
Ephemeroptera	Isonychiidae	Isonychia	0.0064
Diptera	Chironomidae	na	0.0068
Diptera	Chironomidae	na	0.0068
Diptera	Chironomidae	na	0.0068
Diptera	Chironomidae	na	0.0068
Coleoptera	Elmidae	Optioservus	0.0080
Diptera	Chironomidae	na	0.0106
Diptera	Chironomidae	na	0.0106
Diptera	Chironomidae	na	0.0106
Diptera	Chironomidae	na	0.0106
Diptera	Chironomidae	na	0.0106
Diptera	Chironomidae	na	0.0106
Diptera	Chironomidae	na	0.0106
Diptera	Chironomidae	na	0.0106
Diptera	Chironomidae	na	0.0106
Diptera	Chironomidae	na	0.0106
Diptera	Chironomidae	na	0.0106
Coleoptera	Elmidae	Optioservus	0.0109
Coleoptera	Elmidae	Dubiraphia	0.0144
Diptera	Chironomidae	na	0.0154
Diptera	Chironomidae	na	0.0154
Diptera	Chironomidae	na	0.0154
Diptera	Chironomidae	na	0.0154
Diptera	Chironomidae	na	0.0154
Diptera	Chironomidae	na	0.0154
Diptera	Chironomidae	na	0.0154
Diptera	Chironomidae	na	0.0154
Diptera	Chironomidae	na	0.0154
Diptera	Chironomidae	na	0.0154

Coleoptera	Elmidae	Optioservus	0.0187
Diptera	Chironomidae	na	0.0215
Diptera	Chironomidae	na	0.0215
Diptera	Chironomidae	na	0.0215
Megaloptera	Sialidae	Sialis	0.0229
Diptera	Chironomidae	na	0.0290
Diptera	Chironomidae	na	0.0290
Diptera	Chironomidae	na	0.0290
Diptera	Chironomidae	na	0.0290
Diptera	Chironomidae	na	0.0290
Diptera	Chironomidae	na	0.0290
Diptera	Chironomidae	na	0.0290
Diptera	Chironomidae	na	0.0290
Diptera	Chironomidae	na	0.0290
Diptera	Chironomidae	na	0.0290
Ephemeroptera	Ephemerellidae	Attenella	0.0345
Diptera	Chironomidae	na	0.0381
Diptera	Chironomidae	na	0.0381
Diptera	Chironomidae	na	0.0381
Megaloptera	Sialidae	Sialis	0.0425
Trichoptera	Hydropsychidae	Hydropsyche	0.0463
Diptera	Chironomidae	na	0.0487
Diptera	Chironomidae	na	0.0487
Diptera	Chironomidae	na	0.0487
Diptera	Chironomidae	na	0.0487
Diptera	Chironomidae	na	0.0487
Plecoptera	Perlodidae	Isoperla	0.0609
Diptera	Chironomidae	na	0.0611
Diptera	Chironomidae	na	0.0611
Diptera	Chironomidae	na	0.0611
Ephemeroptera	Caenidae	Caenis	0.0634
Megaloptera	Sialidae	Sialis	0.0709
Ephemeroptera	Heptageniidae	Maccaffertium	0.0737
Diptera	Chironomidae	na	0.0754
Coleoptera	Psephenidae	Psephenus	0.1134
Diptera	Chironomidae	na	0.1308
Megaloptera	Sialidae	Sialis	0.1464
Odonata	Gomphidae	Gomphus	0.1492
Ephemeroptera	Baetidae	Procloeon	0.1625
Plecoptera	Leuctridae	Leuctra	0.1656
Diptera	Chironomidae	na	0.1792

Trichoptera	Hydropsychidae	Hydropsyche	0.1884
Megaloptera	Sialidae	Sialis	0.1905
Megaloptera	Sialidae	Sialis	0.1905
Diptera	Chironomidae	na	0.2073
Diptera	Chironomidae	na	0.2073
Diptera	Chironomidae	na	0.2073
Diptera	Chironomidae	na	0.2073
Diptera	Chironomidae	na	0.2073
Diptera	Chironomidae	na	0.2073
Diptera	Chironomidae	na	0.2073
Diptera	Chironomidae	na	0.2073
Diptera	Chironomidae	na	0.2073
Diptera	Chironomidae	na	0.2073
Diptera	Chironomidae	na	0.2073
Diptera	Chironomidae	na	0.2073
Diptera	Chironomidae	na	0.2073
Diptera	Chironomidae	na	0.2073
Ephemeroptera	Baetidae	Procloeon	0.2257
Ephemeroptera	Baetidae	Procloeon	0.2257
Diptera	Chironomidae	na	0.2380
Diptera	Chironomidae	na	0.2380
Diptera	Chironomidae	na	0.2380
Diptera	Chironomidae	na	0.2380
Diptera	Chironomidae	na	0.2380
Diptera	Chironomidae	na	0.2380
Diptera	Chironomidae	na	0.2380
Diptera	Chironomidae	na	0.2380
Diptera	Chironomidae	na	0.2380
Diptera	Chironomidae	na	0.2380
Plecoptera	Leuctridae	Leuctra	0.2472
Plecoptera	Leuctridae	Leuctra	0.2713
Diptera	Chironomidae	na	0.2716
Diptera	Chironomidae	na	0.2716
Diptera	Chironomidae	na	0.2716
Diptera	Chironomidae	na	0.2716
Diptera	Chironomidae	na	0.2716
Diptera	Chironomidae	na	0.2716
Diptera	Chironomidae	na	0.2716
Diptera	Chironomidae	na	0.2716
Diptera	Chironomidae	na	0.2716
Diptera	Chironomidae	na	0.2716

Diptera	Chironomidae	na	0.3080
Diptera	Chironomidae	na	0.3080
Diptera	Chironomidae	na	0.3080
Diptera	Chironomidae	na	0.3080
Diptera	Chironomidae	na	0.3080
Diptera	Chironomidae	na	0.3476
Odonata	Coenagrionidae	Argia	0.4276
Diptera	Chironomidae	na	0.5380
Ephemeroptera	Ephemerellidae	Attenella	1.4053
Ephemeroptera	Heptageniidae	Maccaffertium	2.1527
Ephemeroptera	Heptageniidae	Maccaffertium	4.4113
Trichoptera	Pteronarcyidae	Pteronarcys	78.7555

.

ective dry mass (mg) estimates.

Benthic invertebrate abundance within each log_2 size bin (Slaunch Fork, WV; May 2015 samples).

Counts within each size bin were converted to density estimates by accounting for the total sampling

Log_2 Size Bin - Lower Bound (mg)	Log_2 Size Bin - Upper Bound (mg)	Count per Size Bin
0.0032	0.0064	8
0.0064	0.0128	17
0.0128	0.0256	16
0.0256	0.0512	21
0.0512	0.1024	8
0.1024	0.2048	10
0.2048	0.4096	44
0.4096	0.8192	2
0.8192	1.6384	1
1.6384	3.2768	1
3.2768	6.5536	1
6.5536	13.1072	0
13.1072	26.2144	0
26.2144	52.4288	0
52.4288	104.8576	1

area (0.088 m^2 /Hess sample x 6 Hess samples).

Density (per m^2)
15.15
32.20
30.30
39.77
15.15
18.94
83.33
3.79
1.89
1.89
1.89
0.00
0.00
0.00
1.89

These are the raw fish data from Slaunch Fork, West Virginia (May 2015 samples).

Individuals captured in each of three depletion runs are shown in the table below, with individual dry mass (mg)

RUN	SPECIES	DRY_MASS_MG
Run 1	White sucker	23933
Run 1	Blacknose dace	767
Run 1	Blacknose dace	430
Run 1	Blacknose dace	502
Run 1	Blacknose dace	801
Run 1	Blacknose dace	555
Run 1	Blacknose dace	307
Run 1	Blacknose dace	184
Run 1	Blacknose dace	345
Run 1	Blacknose dace	211
Run 1	Blacknose dace	528
Run 1	Blacknose dace	211
Run 1	Blacknose dace	148
Run 1	Blacknose dace	528
Run 1	Blacknose dace	69
Run 1	Rosyside dace	949
Run 1	Rosyside dace	1029
Run 1	Rosyside dace	949
Run 1	Creek chub	2300
Run 1	Creek chub	4032
Run 1	Creek chub	3730
Run 1	Creek chub	2230
Run 1	Creek chub	2999
Run 1	Creek chub	1113
Run 1	Creek chub	702
Run 1	Creek chub	988
Run 1	Creek chub	148
Run 1	Banded darter	365
Run 1	Creek chub	9223
Run 1	Creek chub	1779
Run 1	Creek chub	4460
Run 1	Creek chub	2300
Run 1	Creek chub	1663
Run 1	Creek chub	3084
Run 1	Stoneroller	1663
Run 1	Mottled sculpin	873
Run 1	Blacknose dace	159
Run 1	Blacknose dace	407

Run 1	Blacknose dace	611
Run 1	Blacknose dace	801
Run 1	Blacknose dace	159
Run 1	Blacknose dace	256
Run 1	Blacknose dace	386
Run 1	Blacknose dace	767
Run 1	Blacknose dace	528
Run 1	Blacknose dace	453
Run 1	Blacknose dace	453
Run 1	Blacknose dace	1157
Run 1	Blacknose dace	988
Run 1	Rosyside dace	767
Run 1	Rosyside dace	910
Run 1	Rosyside dace	555
Run 1	Creek chub	611
Run 1	Creek chub	83
Run 1	Creek chub	2094
Run 1	Creek chub	4032
Run 1	River chub	6339
Run 1	White sucker	6063
Run 1	Rosyside dace	1113
Run 1	Rosyside dace	1345
Run 1	Rosyside dace	1720
Run 1	Rosyside dace	988
Run 1	Rosyside dace	702
Run 1	Creek chub	2833
Run 1	Creek chub	910
Run 1	Creek chub	1720
Run 1	Creek chub	240
Run 1	Stoneroller	2028
Run 1	Mottled sculpin	528
Run 1	Mottled sculpin	1446
Run 1	Mottled sculpin	555
Run 1	Mottled sculpin	256
Run 1	Mottled sculpin	528
Run 1	Banded darter	407
Run 1	Creek chub	211
Run 1	Creek chub	184
Run 1	Blacknose dace	670
Run 1	Blacknose dace	197
Run 1	Blacknose dace	640
Run 1	Blacknose dace	582
Run 1	Blacknose dace	502

Run 1	Blacknose dace	767
Run 1	Blacknose dace	949
Run 1	Blacknose dace	767
Run 1	Blacknose dace	582
Run 1	Blacknose dace	611
Run 1	Blacknose dace	453
Run 1	Blacknose dace	670
Run 1	Blacknose dace	197
Run 1	Blacknose dace	801
Run 1	Blacknose dace	272
Run 1	Blacknose dace	611
Run 1	Blacknose dace	453
Run 1	Blacknose dace	611
Run 1	Blacknose dace	148
Run 1	Blacknose dace	108
Run 1	Blacknose dace	837
Run 1	Blacknose dace	582
Run 1	Blacknose dace	555
Run 1	Rosyside dace	837
Run 1	Rosyside dace	949
Run 1	Rosyside dace	1498
Run 1	Rosyside dace	1663
Run 1	Rosyside dace	1296
Run 1	Rosyside dace	910
Run 1	Rosyside dace	949
Run 1	Rosyside dace	767
Run 1	Rosyside dace	640
Run 1	Creek chub	988
Run 1	Creek chub	2161
Run 1	Creek chub	2094
Run 1	Creek chub	1607
Run 1	Creek chub	1720
Run 1	Creek chub	949
Run 1	Creek chub	1901
Run 1	Creek chub	430
Run 1	Creek chub	767
Run 1	Creek chub	171
Run 1	Fantail darter	555
Run 1	Banded darter	184
Run 1	White sucker	5159
Run 1	Northern hog sucker	7531
Run 1	Mottled sculpin	611
Run 1	Mottled sculpin	837

Run 1	Mottled sculpin	801
Run 1	Mottled sculpin	289
Run 1	Mottled sculpin	801
Run 1	Blacknose dace	108
Run 1	Blacknose dace	528
Run 1	Blacknose dace	767
Run 1	Blacknose dace	988
Run 1	Blacknose dace	949
Run 1	Blacknose dace	345
Run 1	Blacknose dace	148
Run 1	Blacknose dace	611
Run 1	Blacknose dace	197
Run 1	Blacknose dace	225
Run 1	Blacknose dace	211
Run 1	Blacknose dace	184
Run 1	Blacknose dace	767
Run 1	Blacknose dace	272
Run 1	Rosyside dace	611
Run 1	Creek chub	988
Run 1	Creek chub	171
Run 1	Creek chub	6481
Run 1	Creek chub	4032
Run 1	Creek chub	3929
Run 1	Creek chub	407
Run 1	Creek chub	1498
Run 1	Creek chub	8691
Run 1	Banded darter	127
Run 1	Mottled sculpin	734
Run 1	Mottled sculpin	528
Run 1	Mottled sculpin	582
Run 1	Mottled sculpin	1446
Run 1	Mottled sculpin	453
Run 1	Mottled sculpin	477
Run 1	Mottled sculpin	502
Run 1	Mottled sculpin	582
Run 1	Mottled sculpin	1157
Run 1	Mottled sculpin	197
Run 1	Mottled sculpin	670
Run 1	Mottled sculpin	801
Run 1	Mottled sculpin	837
Run 1	Mottled sculpin	640
Run 1	Mottled sculpin	1157
Run 1	Mottled sculpin	272

Run 1	Mottled sculpin	555
Run 1	Rosyside dace	1394
Run 1	Rosyside dace	1345
Run 1	Creek chub	477
Run 1	Creek chub	307
Run 1	Blacknose dace	1157
Run 1	Blacknose dace	640
Run 1	Blacknose dace	1202
Run 1	Blacknose dace	670
Run 1	Blacknose dace	407
Run 1	Blacknose dace	582
Run 1	Blacknose dace	272
Run 1	Creek chub	6770
Run 1	Creek chub	7374
Run 1	Creek chub	1157
Run 1	Creek chub	2094
Run 1	Creek chub	171
Run 1	Rosyside dace	1248
Run 1	Rosyside dace	949
Run 1	Rosyside dace	767
Run 1	Blacknose dace	873
Run 1	Blacknose dace	640
Run 1	Blacknose dace	734
Run 1	Blacknose dace	326
Run 1	Blacknose dace	1113
Run 1	Blacknose dace	528
Run 1	Blacknose dace	171
Run 1	Blacknose dace	767
Run 1	Blacknose dace	430
Run 1	Blacknose dace	528
Run 1	Blacknose dace	211
Run 1	Johnny darter	197
Run 1	Banded darter	211
Run 1	Creek chub	7851
Run 1	Creek chub	6770
Run 1	Creek chub	949
Run 1	Mottled sculpin	801
Run 1	Mottled sculpin	407
Run 1	Mottled sculpin	555
Run 1	Mottled sculpin	801
Run 1	Mottled sculpin	555
Run 1	Mottled sculpin	197
Run 1	Mottled sculpin	430

Run 1	Mottled sculpin	407
Run 1	Mottled sculpin	582
Run 1	Mottled sculpin	1248
Run 1	Creek chub	3084
Run 1	Blacknose dace	767
Run 1	Blacknose dace	197
Run 1	Blacknose dace	734
Run 1	Blacknose dace	949
Run 1	Blacknose dace	528
Run 1	Blacknose dace	289
Run 1	Blacknose dace	159
Run 1	Blacknose dace	272
Run 1	Blacknose dace	670
Run 1	Blacknose dace	502
Run 1	Blacknose dace	873
Run 1	Johnny darter	148
Run 1	Rosyside dace	670
Run 1	Rosyside dace	837
Run 1	Rosyside dace	873
Run 1	Rosyside dace	988
Run 1	Rosyside dace	988
Run 1	Rosyside dace	1202
Run 1	Blacknose dace	670
Run 1	Blacknose dace	502
Run 1	Blacknose dace	272
Run 1	Blacknose dace	256
Run 1	Blacknose dace	184
Run 1	Blacknose dace	386
Run 1	Blacknose dace	225
Run 1	Johnny darter	256
Run 1	Greenside darter	1113
Run 1	Johnny darter	386
Run 1	Mottled sculpin	1779
Run 1	Mottled sculpin	477
Run 1	Mottled sculpin	477
Run 1	Mottled sculpin	477
Run 1	Mottled sculpin	2028
Run 1	Mottled sculpin	1552
Run 1	Mottled sculpin	767
Run 1	Mottled sculpin	256
Run 1	Mottled sculpin	240
Run 1	Mottled sculpin	528
Run 1	Mottled sculpin	611

Run 1	Mottled sculpin	555
Run 1	Mottled sculpin	670
Run 1	Mottled sculpin	949
Run 1	Mottled sculpin	1394
Run 1	Mottled sculpin	670
Run 1	Mottled sculpin	837
Run 1	Mottled sculpin	910
Run 1	Mottled sculpin	873
Run 1	Mottled sculpin	477
Run 1	Mottled sculpin	949
Run 1	Mottled sculpin	430
Run 1	Mottled sculpin	767
Run 1	Mottled sculpin	555
Run 1	Mottled sculpin	326
Run 1	Mottled sculpin	386
Run 1	Mottled sculpin	528
Run 1	Mottled sculpin	453
Run 1	Creek chub	2230
Run 1	Rosyside dace	910
Run 1	Rosyside dace	1498
Run 1	Rosyside dace	767
Run 1	Blacknose dace	1070
Run 1	Blacknose dace	640
Run 1	Blacknose dace	555
Run 1	Blacknose dace	611
Run 1	Blacknose dace	171
Run 1	Blacknose dace	477
Run 1	Blacknose dace	767
Run 1	Blacknose dace	670
Run 1	Blacknose dace	148
Run 1	Creek chub	5407
Run 1	Creek chub	1345
Run 1	Stoneroller	2300
Run 1	Fantail darter	640
Run 1	Blacknose dace	386
Run 1	Blacknose dace	477
Run 1	Blacknose dace	127
Run 1	Blacknose dace	148
Run 1	Johnny darter	148
Run 1	Rosyside dace	837
Run 1	Rosyside dace	910
Run 1	Rosyside dace	1394
Run 1	Mottled sculpin	670

Run 1	Mottled sculpin	640
Run 1	Mottled sculpin	1029
Run 1	Mottled sculpin	345
Run 1	Mottled sculpin	988
Run 1	Mottled sculpin	873
Run 1	Mottled sculpin	528
Run 1	Mottled sculpin	502
Run 1	Mottled sculpin	528
Run 1	Mottled sculpin	910
Run 1	Mottled sculpin	453
Run 1	Mottled sculpin	528
Run 1	Mottled sculpin	502
Run 1	Mottled sculpin	582
Run 1	Blacknose dace	184
Run 1	Creek chub	1248
Run 1	Creek chub	1248
Run 1	Creek chub	1157
Run 1	Creek chub	1029
Run 1	Creek chub	988
Run 1	Creek chub	477
Run 1	Creek chub	345
Run 1	Creek chub	734
Run 1	Creek chub	1498
Run 1	Creek chub	1113
Run 1	Creek chub	1157
Run 1	Creek chub	837
Run 1	Creek chub	430
Run 1	Blacknose dace	137
Run 1	Johnny darter	184
Run 1	Blacknose dace	555
Run 1	Blacknose dace	453
Run 1	Blacknose dace	345
Run 1	Blacknose dace	386
Run 1	Blacknose dace	555
Run 1	Blacknose dace	477
Run 1	Blacknose dace	225
Run 1	Blacknose dace	386
Run 1	Blacknose dace	502
Run 1	Blacknose dace	582
Run 1	Blacknose dace	211
Run 1	Mottled sculpin	910
Run 1	Mottled sculpin	1498
Run 1	Mottled sculpin	1607

Run 1	Mottled sculpin	767
Run 1	Mottled sculpin	988
Run 1	Mottled sculpin	430
Run 1	Mottled sculpin	949
Run 1	Mottled sculpin	453
Run 1	Mottled sculpin	528
Run 1	Mottled sculpin	734
Run 1	Mottled sculpin	767
Run 1	Blacknose dace	582
Run 1	Northern hog sucker	17138
Run 1	Greenside darter	767
Run 1	Blacknose dace	734
Run 1	Blacknose dace	148
Run 1	Fantail darter	1345
Run 1	Blacknose dace	345
Run 1	Blacknose dace	184
Run 1	Blacknose dace	611
Run 1	Blacknose dace	184
Run 1	Blacknose dace	307
Run 1	Blacknose dace	272
Run 1	Blacknose dace	528
Run 1	Blacknose dace	256
Run 1	Blacknose dace	211
Run 1	Blacknose dace	272
Run 1	Blacknose dace	159
Run 1	Blacknose dace	159
Run 1	Blacknose dace	670
Run 1	Blacknose dace	211
Run 1	Blacknose dace	225
Run 1	Blacknose dace	256
Run 1	Blacknose dace	184
Run 1	Blacknose dace	184
Run 1	Blacknose dace	171
Run 1	Blacknose dace	837
Run 1	Blacknose dace	555
Run 1	Blacknose dace	197
Run 1	Blacknose dace	611
Run 1	Blacknose dace	837
Run 1	Blacknose dace	211
Run 1	Blacknose dace	148
Run 1	Blacknose dace	211
Run 1	Blacknose dace	171
Run 1	Blacknose dace	502

Run 1	Fantail darter	345
Run 1	Rosyside dace	225
Run 1	Rosyside dace	345
Run 1	Rosyside dace	184
Run 1	Rosyside dace	307
Run 1	Creek chub	345
Run 1	Creek chub	582
Run 1	Creek chub	240
Run 1	Creek chub	837
Run 1	Creek chub	137
Run 1	Rosyside dace	1070
Run 1	Creek chub	767
Run 1	Creek chub	949
Run 1	Blacknose dace	91
Run 1	Blacknose dace	272
Run 1	Blacknose dace	159
Run 1	Blacknose dace	767
Run 1	Blacknose dace	197
Run 1	Blacknose dace	184
Run 1	Blacknose dace	211
Run 1	Blacknose dace	240
Run 1	Blacknose dace	159
Run 1	Blacknose dace	611
Run 1	Blacknose dace	148
Run 1	Blacknose dace	184
Run 1	Blacknose dace	171
Run 1	Blacknose dace	171
Run 1	Blacknose dace	345
Run 1	Blacknose dace	256
Run 1	Mottled sculpin	2752
Run 1	Mottled sculpin	1446
Run 1	Mottled sculpin	670
Run 1	Mottled sculpin	801
Run 1	Mottled sculpin	670
Run 1	Mottled sculpin	670
Run 1	Mottled sculpin	502
Run 1	Mottled sculpin	502
Run 1	Mottled sculpin	407
Run 1	Mottled sculpin	801
Run 1	Mottled sculpin	801
Run 1	Mottled sculpin	345
Run 1	Mottled sculpin	734
Run 1	Mottled sculpin	988

Run 1	Mottled sculpin	528
Run 1	Mottled sculpin	211
Run 1	Mottled sculpin	1157
Run 1	Mottled sculpin	988
Run 1	Mottled sculpin	211
Run 1	Mottled sculpin	1113
Run 1	Mottled sculpin	453
Run 1	Mottled sculpin	873
Run 1	Mottled sculpin	837
Run 1	Mottled sculpin	1029
Run 1	Mottled sculpin	528
Run 2	Creek chub	3171
Run 2	Creek chub	1345
Run 2	Blacknose dace	837
Run 2	Northern hog sucker	10157
Run 2	Rosyside dace	837
Run 2	Blacknose dace	345
Run 2	Blacknose dace	148
Run 2	Mottled sculpin	910
Run 2	Mottled sculpin	365
Run 2	Mottled sculpin	734
Run 2	Creek chub	801
Run 2	Creek chub	988
Run 2	Creek chub	949
Run 2	Creek chub	197
Run 2	Blacknose dace	734
Run 2	Blacknose dace	159
Run 2	Blacknose dace	225
Run 2	Blacknose dace	184
Run 2	Stoneroller	767
Run 2	Rosyside dace	1113
Run 2	Johnny darter	240
Run 2	Blacknose dace	184
Run 2	Mottled sculpin	1113
Run 2	Mottled sculpin	184
Run 2	Mottled sculpin	289
Run 2	Mottled sculpin	670
Run 2	Mottled sculpin	670
Run 2	Mottled sculpin	949
Run 2	Mottled sculpin	289
Run 2	White sucker	12214
Run 2	Northern hog sucker	7851
Run 2	Creek chub	2519

Run 2	Creek chub	1202
Run 2	Creek chub	289
Run 2	Creek chub	407
Run 2	Blacknose dace	477
Run 2	Blacknose dace	407
Run 2	Blacknose dace	159
Run 2	Blacknose dace	184
Run 2	Blacknose dace	171
Run 2	Blacknose dace	171
Run 2	Rosyside dace	1446
Run 2	Mottled sculpin	801
Run 2	Mottled sculpin	949
Run 2	Mottled sculpin	988
Run 2	Mottled sculpin	148
Run 2	Mottled sculpin	386
Run 2	Mottled sculpin	988
Run 2	Mottled sculpin	640
Run 2	Mottled sculpin	910
Run 2	Mottled sculpin	801
Run 2	Mottled sculpin	611
Run 2	Mottled sculpin	640
Run 2	Mottled sculpin	611
Run 2	Mottled sculpin	528
Run 2	Mottled sculpin	555
Run 2	Blacknose dace	184
Run 2	Blacknose dace	211
Run 2	Blacknose dace	171
Run 2	Blacknose dace	184
Run 2	Blacknose dace	801
Run 2	Blacknose dace	582
Run 2	Blacknose dace	197
Run 2	Blacknose dace	197
Run 2	Blacknose dace	99
Run 2	Rosyside dace	767
Run 2	Rosyside dace	1394
Run 2	Mottled sculpin	1394
Run 2	Mottled sculpin	1070
Run 2	Mottled sculpin	555
Run 2	Mottled sculpin	988
Run 2	Mottled sculpin	1248
Run 2	Mottled sculpin	555
Run 2	Mottled sculpin	1157
Run 2	Blacknose dace	734

Run 2	Blacknose dace	184
Run 2	Blacknose dace	184
Run 2	Creek chub	949
Run 2	Creek chub	1070
Run 2	Johnny darter	326
Run 2	Stoneroller	3537
Run 2	Rosyside dace	767
Run 2	Rosyside dace	910
Run 2	Rosyside dace	910
Run 2	Blacknose dace	949
Run 2	Blacknose dace	272
Run 2	Mottled sculpin	1446
Run 2	Mottled sculpin	1663
Run 2	Mottled sculpin	477
Run 2	Mottled sculpin	949
Run 2	Mottled sculpin	582
Run 2	Mottled sculpin	611
Run 2	Mottled sculpin	988
Run 2	Mottled sculpin	1552
Run 2	Mottled sculpin	365
Run 2	Mottled sculpin	1070
Run 2	Mottled sculpin	873
Run 2	Mottled sculpin	453
Run 2	Mottled sculpin	1070
Run 2	Mottled sculpin	1029
Run 2	Mottled sculpin	670
Run 2	Mottled sculpin	477
Run 2	Mottled sculpin	430
Run 2	Mottled sculpin	702
Run 2	Fantail darter	801
Run 2	Creek chub	670
Run 2	Creek chub	1157
Run 2	Mottled sculpin	611
Run 2	Mottled sculpin	1446
Run 2	Mottled sculpin	582
Run 2	Mottled sculpin	767
Run 2	Mottled sculpin	949
Run 2	Blacknose dace	1029
Run 2	Blacknose dace	117
Run 2	Blacknose dace	171
Run 2	Stoneroller	582
Run 2	Rosyside dace	1248
Run 2	Creek chub	5282

Run 2	Rosyside dace	1394
Run 2	Banded darter	197
Run 2	Blacknose dace	407
Run 2	Banded darter	453
Run 2	Creek chub	611
Run 2	Creek chub	502
Run 2	Mottled sculpin	640
Run 2	Mottled sculpin	611
Run 2	Mottled sculpin	734
Run 2	Mottled sculpin	477
Run 2	Mottled sculpin	453
Run 2	Creek chub	12657
Run 2	Banded darter	197
Run 2	Mottled sculpin	386
Run 2	Mottled sculpin	289
Run 2	Mottled sculpin	801
Run 2	Mottled sculpin	407
Run 2	Blacknose dace	528
Run 2	Blacknose dace	159
Run 2	Blacknose dace	225
Run 2	Banded darter	171
Run 2	Creek chub	326
Run 2	Creek chub	1498
Run 2	Creek chub	1498
Run 2	Creek chub	386
Run 2	Mottled sculpin	453
Run 2	Blacknose dace	611
Run 2	Blacknose dace	949
Run 2	Mottled sculpin	611
Run 2	Northern hog sucker	9223
Run 2	Mottled sculpin	767
Run 2	Mottled sculpin	837
Run 2	Mottled sculpin	240
Run 2	Blacknose dace	211
Run 2	Blacknose dace	225
Run 2	Blacknose dace	159
Run 2	Banded darter	240
Run 2	Banded darter	582
Run 2	Mottled sculpin	555
Run 2	Mottled sculpin	1029
Run 2	Mottled sculpin	1345
Run 2	Mottled sculpin	1157
Run 2	Blacknose dace	1157

Run 2	Blacknose dace	611
Run 2	Banded darter	159
Run 2	Banded darter	225
Run 2	Blacknose dace	256
Run 2	Blacknose dace	117
Run 2	Blacknose dace	184
Run 2	Blacknose dace	611
Run 2	Blacknose dace	1394
Run 2	Blacknose dace	184
Run 2	Blacknose dace	159
Run 2	Blacknose dace	611
Run 2	Mottled sculpin	582
Run 2	Creek chub	20654
Run 3	White sucker	5282
Run 3	Blacknose dace	611
Run 3	Blacknose dace	582
Run 3	Creek chub	1779
Run 3	Rosyside dace	345
Run 3	Rosyside dace	1202
Run 3	Mottled sculpin	1113
Run 3	Mottled sculpin	611
Run 3	Mottled sculpin	734
Run 3	Fantail darter	910
Run 3	Rosyside dace	528
Run 3	Rosyside dace	801
Run 3	Rosyside dace	767
Run 3	Rosyside dace	801
Run 3	Johnny darter	307
Run 3	Johnny darter	225
Run 3	Johnny darter	225
Run 3	Mottled sculpin	502
Run 3	Mottled sculpin	1070
Run 3	Mottled sculpin	837
Run 3	Blacknose dace	289
Run 3	Blacknose dace	326
Run 3	Blacknose dace	197
Run 3	Blacknose dace	225
Run 3	Blacknose dace	148
Run 3	Blacknose dace	289
Run 3	Blacknose dace	225
Run 3	Blacknose dace	99
Run 3	Blacknose dace	184
Run 3	Blacknose dace	108

Run 3	Blacknose dace	159
Run 3	Rosyside dace	734
Run 3	Rosyside dace	386
Run 3	Mottled sculpin	949
Run 3	Mottled sculpin	528
Run 3	Mottled sculpin	910
Run 3	Mottled sculpin	949
Run 3	Mottled sculpin	326
Run 3	White sucker	5282
Run 3	Blacknose dace	611
Run 3	Blacknose dace	582
Run 3	Creek chub	1779
Run 3	Rosyside dace	345
Run 3	Rosyside dace	1202
Run 3	Mottled sculpin	1113
Run 3	Mottled sculpin	611
Run 3	Mottled sculpin	734
Run 3	Fantail darter	910
Run 3	Rosyside dace	528
Run 3	Rosyside dace	801
Run 3	Rosyside dace	767
Run 3	Rosyside dace	801
Run 3	Johnny darter	307
Run 3	Johnny darter	225
Run 3	Johnny darter	225
Run 3	Mottled sculpin	502
Run 3	Mottled sculpin	1070
Run 3	Mottled sculpin	837
Run 3	Blacknose dace	289
Run 3	Blacknose dace	326
Run 3	Blacknose dace	197
Run 3	Blacknose dace	225
Run 3	Blacknose dace	148
Run 3	Blacknose dace	289
Run 3	Blacknose dace	225
Run 3	Blacknose dace	99
Run 3	Blacknose dace	184
Run 3	Blacknose dace	108
Run 3	Blacknose dace	159
Run 3	Rosyside dace	734
Run 3	Rosyside dace	386
Run 3	Mottled sculpin	949
Run 3	Mottled sculpin	528

Run 3	Mottled sculpin	910
Run 3	Mottled sculpin	949
Run 3	Mottled sculpin	326
Run 3	White sucker	5282
Run 3	Blacknose dace	611
Run 3	Blacknose dace	582
Run 3	Creek chub	1779
Run 3	Rosyside dace	345
Run 3	Rosyside dace	1202
Run 3	Mottled sculpin	1113
Run 3	Mottled sculpin	611
Run 3	Mottled sculpin	734
Run 3	Fantail darter	910
Run 3	Rosyside dace	528
Run 3	Rosyside dace	801
Run 3	Rosyside dace	767
Run 3	Rosyside dace	801
Run 3	Johnny darter	307
Run 3	Johnny darter	225
Run 3	Johnny darter	225
Run 3	Mottled sculpin	502
Run 3	Mottled sculpin	1070
Run 3	Mottled sculpin	837
Run 3	Blacknose dace	289
Run 3	Blacknose dace	326
Run 3	Blacknose dace	197
Run 3	Blacknose dace	225
Run 3	Blacknose dace	148
Run 3	Blacknose dace	289
Run 3	Blacknose dace	225
Run 3	Blacknose dace	99
Run 3	Blacknose dace	184
Run 3	Blacknose dace	108
Run 3	Blacknose dace	159
Run 3	Rosyside dace	734
Run 3	Rosyside dace	386
Run 3	Mottled sculpin	949
Run 3	Mottled sculpin	528
Run 3	Mottled sculpin	910
Run 3	Mottled sculpin	949
Run 3	Mottled sculpin	326
Run 3	White sucker	5282
Run 3	Blacknose dace	611

Run 3	Blacknose dace	582
Run 3	Creek chub	1779
Run 3	Rosyside dace	345
Run 3	Rosyside dace	1202
Run 3	Mottled sculpin	1113
Run 3	Mottled sculpin	611
Run 3	Mottled sculpin	734
Run 3	Fantail darter	910
Run 3	Rosyside dace	528
Run 3	Rosyside dace	801
Run 3	Rosyside dace	767
Run 3	Rosyside dace	801
Run 3	Johnny darter	307
Run 3	Johnny darter	225
Run 3	Johnny darter	225
Run 3	Mottled sculpin	502
Run 3	Mottled sculpin	1070
Run 3	Mottled sculpin	837
Run 3	Blacknose dace	289
Run 3	Blacknose dace	326
Run 3	Blacknose dace	197
Run 3	Blacknose dace	225
Run 3	Blacknose dace	148
Run 3	Blacknose dace	289
Run 3	Blacknose dace	225
Run 3	Blacknose dace	99
Run 3	Blacknose dace	184
Run 3	Blacknose dace	108
Run 3	Blacknose dace	159
Run 3	Rosyside dace	734
Run 3	Rosyside dace	386
Run 3	Mottled sculpin	949
Run 3	Mottled sculpin	528
Run 3	Mottled sculpin	910
Run 3	Mottled sculpin	949
Run 3	Mottled sculpin	326

estimates.

Example Zippin calculation for the 838.86 - 1677.72 mg log_2 size bin.

All variables listed below are consistent with Equations 1 and 2 from the main article and from Lo
Iterative potential values of ***n*** *are examined (i.e. filled down in Column K) to check the correspon*
When the optimal ***n*** *estimate has been identified (as indicated by the largest Integral value that*

Number of fishes captured								
Pass 1	**Pass 2**	**Pass 3**	***T***	***k***	***X***	**solution-1**	**solution-2**	**solution-3**
90	47	28	165	3	227	166.0000	0.3897	0.3875
90	47	28	165	3	227	28.5000	0.4216	0.4196
90	47	28	165	3	227	16.0000	0.4503	0.4485
90	47	28	165	3	227	11.3125	0.4763	0.4747
90	47	28	165	3	227	8.8571	0.5000	0.4985
90	47	28	165	3	227	8.5000	0.5045	0.5030
90	47	28	165	3	227	8.1739	0.5089	0.5074
90	47	28	165	3	227	7.8750	0.5132	0.5118
90	47	28	165	3	227	7.6000	0.5174	0.5160
90	47	28	165	3	227	7.3462	0.5216	0.5202
90	47	28	165	3	227	7.1111	0.5257	0.5244
90	47	28	165	3	227	6.8929	0.5297	0.5284
90	47	28	165	3	227	6.6897	0.5337	0.5324
90	47	28	165	3	227	6.5000	0.5376	0.5363
90	47	28	165	3	227	6.3226	0.5414	0.5402
90	47	28	165	3	227	6.1563	0.5452	0.5440
90	47	28	165	3	227	6.0000	0.5489	0.5477
90	47	28	165	3	227	5.8529	0.5526	0.5514
90	47	28	165	3	227	5.7143	0.5561	0.5550
90	47	28	165	3	227	5.5833	0.5597	0.5585

ckwood and Schneider[38].

ding Integral values.

is less than or equal to 1.0), it must be converted to a density (per m^2) estimate using the total st

solution-4	*n*	Integral	
0.3852	165	9.655	
0.4175	170	2.105	
0.4467	175	1.443	
0.4730	180	1.210	
0.4970	185	1.097	
0.5015	186	1.082	
0.5060	187	1.068	
0.5103	188	1.055	
0.5146	189	1.044	
0.5188	190	1.034	
0.5230	191	1.025	
0.5271	192	1.017	
0.5311	193	1.009	
0.5350	194	1.003	
0.5389	**195**	**0.996**	**This is the largest integral value that is less than or equal to 1.*
0.5427	196	0.991	
0.5464	197	0.986	
0.5501	198	0.981	
0.5538	199	0.977	
0.5573	200	0.973	

ream channel survey area.

.0.

Estimated fish abundance within each log_2 size bin (Slaunch Fork, WV; May 2015 samples).

Raw counts from the first, second, and third depletion samples are shown for each size bin, with the
Zippin abundance estimates are converted to per m^2 density estimates by accounting for the total s

Log_2 Size Bin - Lower Bound (mg)	Log_2 Size Bin - Upper Bound (mg)	Count per Size Bin - Run 1
52.4288	104.85759	3
104.8576	209.71519	56
209.7152	419.43039	74
419.4304	838.86079	171
838.8608	1677.72159	90
1677.7216	3355.44319	22
3355.4432	6710.88639	11
6710.8864	13421.77279	7
13421.7728	26843.54559	2

' resulting Zipping abundance estimate.

surface area of surveyed stream channel.

Count per Size Bin - Run 2	Count per Size Bin - Run 3	Zippin Estimated Abundance	Density (per m^2)
1	4	8	0.0038
30	20	129	0.0616
27	44	221	0.1056
59	48	312	0.1491
47	28	195	0.0932
2	4	60	0.0287
2	4	17	0.0081
5	0	12	0.0057
1	0	3	0.0014

Complete formatted benthic invertebrate and fish data from Slaunch For

Normalized density estimates were obtained by dividing the total density e

Log_{10} transformed Mean Dry Mass and Normalized Density estimates are

Log_2 Size Bin - Lower Bound (mg)	Log_2 Size Bin - Upper Bound (mg)
0.0032	0.0064
0.0064	0.0128
0.0128	0.0256
0.0256	0.0512
0.0512	0.1024
0.1024	0.2048
0.2048	0.4096
0.4096	0.8192
0.8192	1.6384
1.6384	3.2768
3.2768	6.5536
52.4288	104.8576
104.8576	209.7152
209.7152	419.4304
419.4304	838.8608
838.8608	1677.7216
1677.7216	3355.4432
3355.4432	6710.8864
6710.8864	13421.7728
13421.7728	26843.5456

k, West Virginia (May 2015 samples).

stimate within each log $_2$ size bin by the width of the respective size bin.

shown in the last two columns; these columns are used to plot and model the size spectrum.

Log_2 Size Bin - Arithmetic Mean Dry Mass (mg)	Log_2 Size Bin - Width (mg)	Taxa
0.0048	0.0032	Invert
0.0096	0.0064	Invert
0.0192	0.0128	Invert
0.0384	0.0256	Invert
0.0768	0.0512	Invert
0.1536	0.1024	Invert
0.3072	0.2048	Invert
0.6144	0.4096	Invert
1.2288	0.8192	Invert
2.4576	1.6384	Invert
4.9152	3.2768	Invert
78.6432	104.8576	Invert
157.2864	209.7152	Fish
314.5728	419.4304	Fish
629.1456	838.8608	Fish
1258.2912	1677.7216	Fish
2516.5824	3355.4432	Fish
5033.1648	6710.8864	Fish
10066.3296	13421.7728	Fish
20132.6592	26843.5456	Fish

Total Density (per m^2)	Normalized Density (per m^2)	Log_{10} Mean Dry Mass (mg)
15.1515	4734.84848485	-2.3188
32.1970	5030.77651515	-2.0177
30.3030	2367.42424242	-1.7167
39.7727	1553.62215909	-1.4157
15.1515	295.92803030	-1.1146
18.9394	184.95501894	-0.8136
83.3333	406.90104167	-0.5126
3.7879	9.24775095	-0.2115
1.8939	2.31193774	0.0895
1.8939	1.15596887	0.3905
1.8939	0.57798443	0.6915
1.8939	0.01806201	1.8957
0.0526	0.00025061	2.1967
0.0750	0.00017884	2.4977
0.1362	0.00016233	2.7988
0.0817	0.00004870	3.0998
0.0134	0.00000399	3.4008
0.0081	0.00000121	3.7018
0.0067	0.00000050	4.0029
0.0019	0.00000007	4.3039

Log_{10} Normalized Density (per m^2)
3.6753
3.7016
3.3743
3.1913
2.4712
2.2671
2.6095
0.9660
0.3640
0.0629
-0.2381
-1.7432
-3.6010
-3.7475
-3.7896
-4.3125
-5.3994
-5.9171
-6.3025
-7.1475

1.1 Isolate fishes within the study reach to create a closed fish assemblage.

1.1.1 Identify the upstream and downstream (direction is relative to a surveyor facing 'upstream' and **against** the water current) ends of the study reach then mark the ends with removable flagging tape.

1.1.2 Measure the width of the wetted stream channel at 5—10 transects, distributed evenly along the length of the study reach. Estimate the total surface area of the study reach as the average wetted channel width multiplied by the total length of the reach.

1.1.3 Secure block nets (i.e., knotless seines with floats on the top line and weights on the bottom line) across the stream channel at the upstream and downstream ends of the study reach.

1.1.3.1 At the UPSTREAM end of the study reach, locate a tree, root, large rock, or other solid object that can be used to anchor a net on each side of the stream. The availability of suitable anchor points on each side of the stream will likely affect the position of the upstream boundary.

1.1.3.2 Select one piece of polypropylene rope and create a loop at each end using a bowline knot. Use ONLY a bowline knot, as other knots may become permanently sealed when exposed to moisture and high tension. For instructions on tying a bowline knot, see **Figure 1**.

1.1.3.3 Wrap the rope around the tree/root/rock and feed the loop at one end through the loop at the other end to create an anchor point (**Figure 2**). Shorten or lengthen the rope anchor by adding or removing wraps around the tree/root/rock.

1.1.3.4 Repeat steps 1.1.3.1—1.1.3.3 to establish a second anchor point on the opposite side of the stream.

1.1.3.5 Create a loop in the lines at each of the four corners of the block net using a bowline knot. Use ONLY a bowline knot (**Figure 1**).

1.1.3.6 Connect both sides of the TOP line of the block net (the line with floats) to the anchor points using cam-action tie-down straps. Insert the hooks at either end of the tie-down strap into the loops at the corners of the block net and the anchor points (**Figure 2**). Pull the free tether of the tie-down strap through the cam buckle to tighten each point of contact.

1.1.3.7 Secure the BOTTOM line of the block net (the line with weights) by pinning them to the stream bank with tent stakes.

1.1.3.8 Establish a seal with the bottom of the stream using large rocks to pin the block net down. Place rocks on the side of the net facing UPSTREAM. Be sure that the top of the net remains above water level (**Figure 2**). Adjust the height(s) of the anchor point(s) as needed.

1.1.3.9 Set a second block net by repeating steps 1.1.3.1—1.1.3.8 at the DOWNSTREAM end of the study reach.

1.2 Perform the first of three fish sampling depletion passes within the enclosed study reach. Our protocol assumes that a backpack electrofisher is available and all surveying crew personnel are properly trained to use it.

1.2.1 Beginning at the DOWNSTREAM end of the enclosed study reach, turn the backpack electrofisher on and move in the upstream direction. Progress slowly, moving side-to-side throughout the study reach to ensure all instream habitats are sampled. The first depletion pass is complete when the upstream net is reached.

1.2.2 Supporting crew members follow the leader (who is operating the electrofisher), collecting stunned fishes with dip nets as they are spotted and transferring them to temporary buckets, then to aerated holding tubs. Use small battery powered 'bait bucket' pumps with aeration stones to ensure that captured fishes remain healthy.

1.3 Process fishes collected in the first depletion pass.

1.3.1 Determine whether anesthesia will be needed. Live fishes are often difficult to handle and sedation may be necessary to minimize stress and injury to fish specimens. If anesthesia is used, two options are widely (as of February 2019) available: Tricaine-S (tricaine methanesulfonate, MS-222) and carbon dioxide (baking soda).

1.3.1.1 When using sedatives, carefully follow all instructions provided with the anesthetic product. In all cases, the anesthetic compound will be mixed in an aerated water bath. Collected fishes will then be submerged in the bath until sedation is observed. Once sedated, fishes must be processed as quickly as possible, as prolonged exposure to sedatives may cause death.

1.3.2 Use small dip nets to retrieve sampled fishes from the holding tank (with or without sedation), individually or in small batches, for identification. Place specimens in white plastic or enamel trays and use forceps and magnifying glasses for examination. Use local or regional identification keys (e.g., "The Fishes of Ohio")[21] to aid in identification.

1.3.3 Measure total length (from tip of snout to end of caudal fin) for each specimen then weigh on a field balance. If using an electronic balance, select one with 0.1 or 0.01 g precision. Keep a transparent plastic box on hand to use, as necessary, as a wind and rain baffle (it must be large enough to cover the balance and specimens being weighed).

1.3.4 Record all information (species identity, total length, and weight) on waterproof data sheets. A printable example of a fish data sheet is provided in **Appendix 1**.

1.3.5 Once processed, return fishes to a separate aerated holding/recovery bin. When all fishes have been processed, release them **downstream** of the downstream block net. (If you accidentally release them into your enclosed study reach, you will ruin your sample!) If anesthesia was used, wait to release until all fishes have recovered and regained equilibrium.

1.4 Perform the second and third depletion passes.

1.4.1 Collect the remaining depletion pass samples by repeating steps 1.2–1.3. Ensure that SAMPLING EFFORT REMAINS CONSISTENT among all three passes. Use the same pace of movement (timing the process is recommended) and same crew members to resurvey the sampling reach.

2.1 Select benthic macroinvertebrate sample sites within the boundaries of the fish sampling reach that are representative of the major types of physical habitats (e.g., riffles or runs) observed in the study reach.

2.2 Using a fixed-area sampler, collect the first benthic macroinvertebrate sample. In shallow streams with extensive gravel-to-pebble size material, the Surber sampler and Hess sampler are the most commonly used devices but any fixed-area sampler can be used.

2.2.1 Place the sampling device firmly against the stream bottom with the sample collection net oriented downstream; move large cobbles as necessary to establish a firm seal with the substrate.

2.2.2 Use a wire or plastic brush to vigorously scrub the substrate within the sampling area for a period of 2 min, allowing dislodged benthic macroinvertebrates to drift into the sample net.

2.2.3 Transfer the sample contents from the net to a plastic jar and cover with 70% isopropyl alcohol for preservation. Label the jar and store it in a safe location for transfer to the lab.

2.3 Collect and preserve additional benthic macroinvertebrate samples, repeating step 2.2.

2.4 Return all collected samples to the lab for processing.