Video Article

Modeling the Size Spectrum for Macroinvertebrates and Fishes in Stream Ecosystems

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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.

Video Link

The video component of this article can be found at https://www.jove.com/video/59945/

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[1,2,3]. 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. **Isolating fishes within the study reach to create a closed fish assemblage**
    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.
    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.
    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. 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.
        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**.
        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.
        4. Repeat steps 1.1.3.1—1.1.3.3 to establish a second anchor point on the opposite side of the stream.
        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**).
        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.
        7. Secure the bottom line of the block net (the line with weights) by pinning them to the stream bank with tent stakes.
        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.
        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.

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. 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.
   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. 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.

3. **Processing fishes collected in the first depletion pass**
   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.
   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.
   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.
   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).
   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**.
   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.

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. 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.
   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.

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

2. Collection and processing of benthic macroinvertebrate samples

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. 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].
   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. 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.
   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.

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.
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.

5. In the lab, sort and identify preserved benthic macroinvertebrate samples.
    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. 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.
    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[25,26,27,28] is available to assist in identification.
    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.
    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

An animation demonstrating how to format the fish and invertebrate data for use in size spectra analysis is available at http://bit.ly/SizeSpectraDensities.

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**.
2. Estimate fish abundance within each of the corresponding size bins.
    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.
    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. Use the Zippin maximum likelihood equation[15,32] to estimate total fish abundance (*n*) within the smallest fish size bin.
        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.
        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. 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**.
    4. Repeat step 3.2.3 for each of the remaining fish size bins.
    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. 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.
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.

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.
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

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**).
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].
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 $\underline{D} \propto \acute{M}$ 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 $\underline{D}$ are variable among streams, with highest densities in Camp Creek (intercept = 0.71) and much lower densities in Cabin Creek (intercept = 0.07).

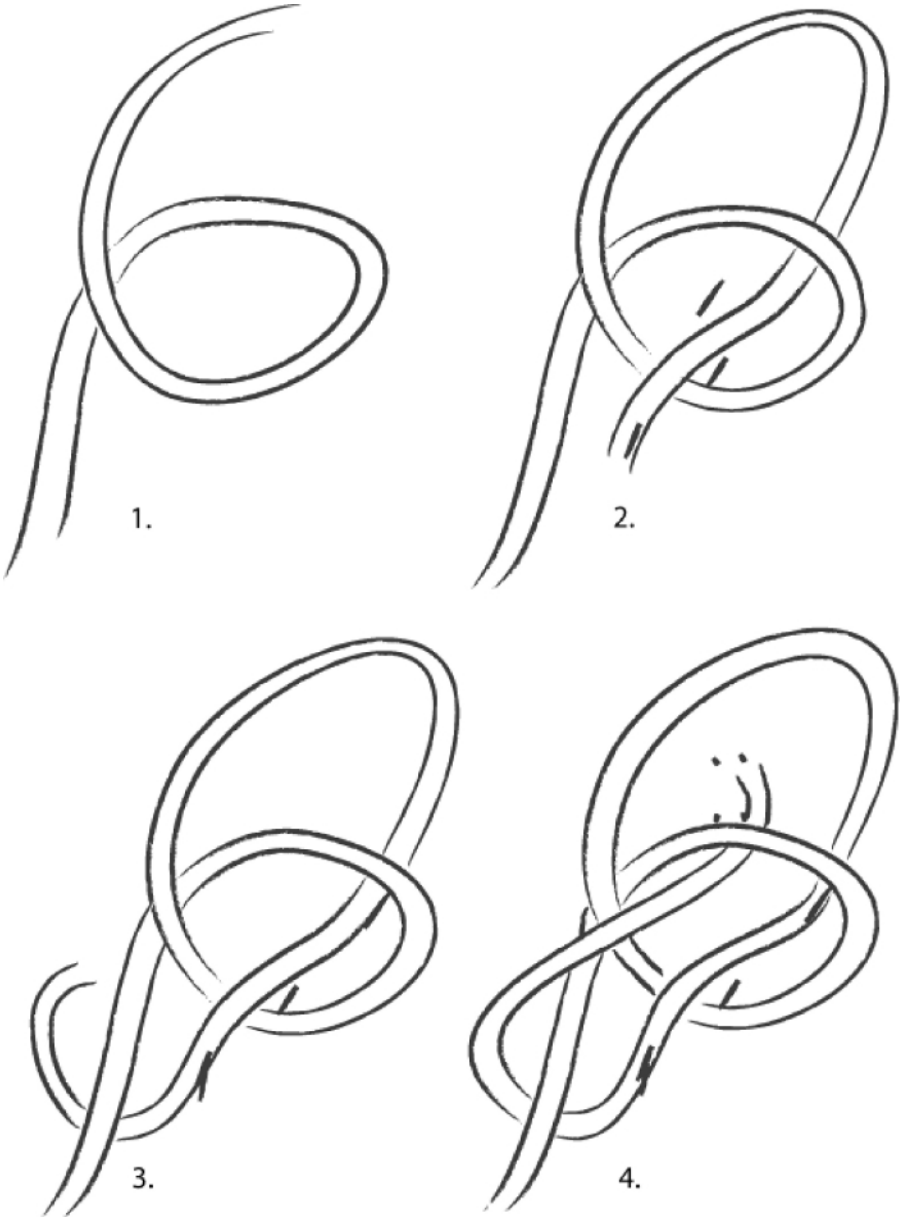


Figure 1. Four-step illustration of the bowline knot.
Original illustration was created by Luis Dantas and is available at https://commons.wikimedia.org/wiki/File:Bowline_in_four_steps.png. This image is freely distributed under a CC-BY-SA-3.0 Creative Commons license (http://creativecommons.org/licenses/by-sa/3.0/).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

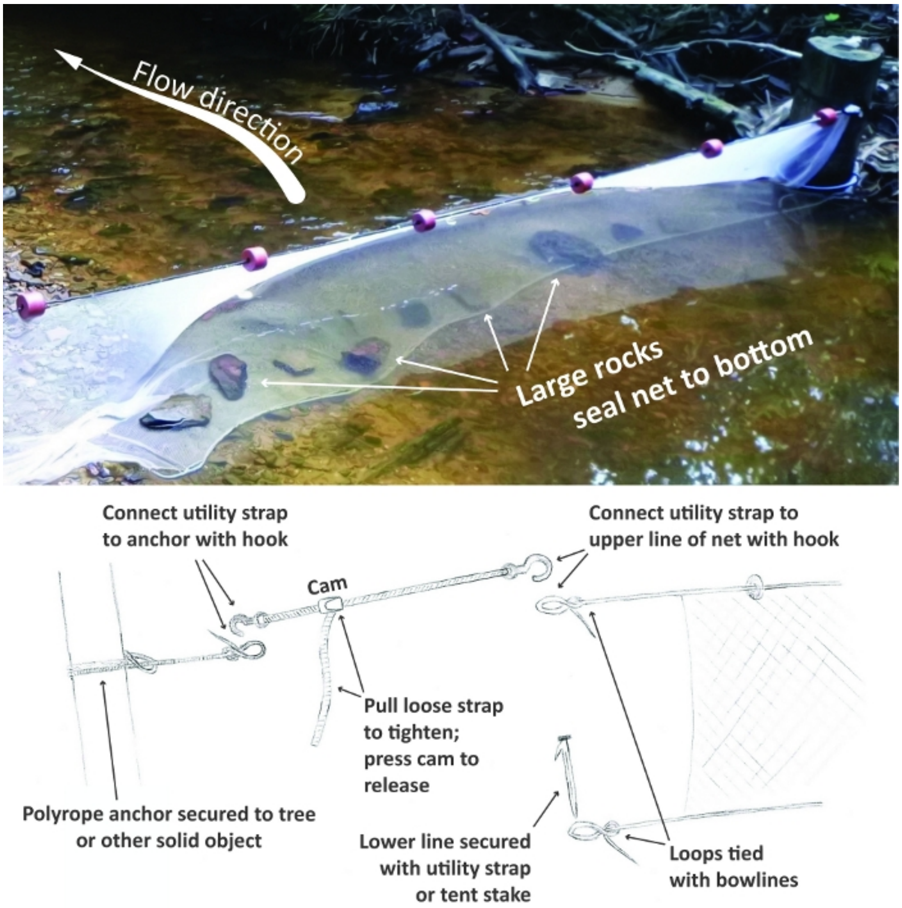
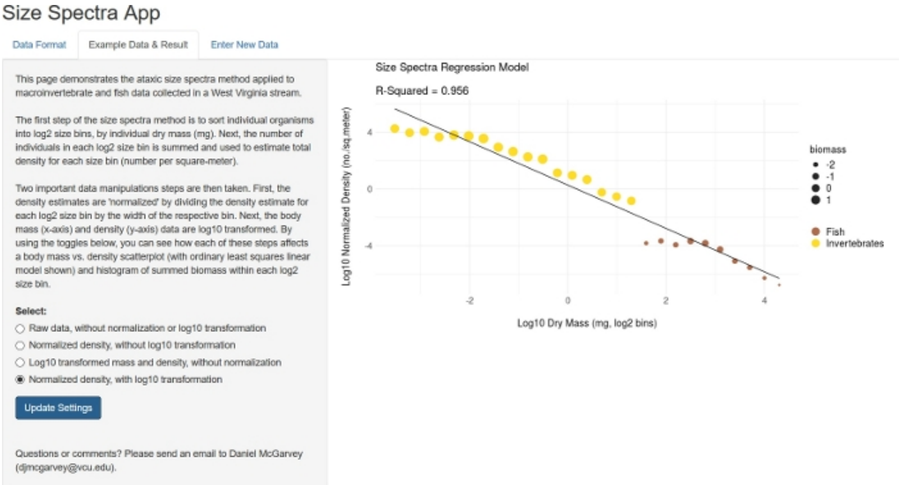


Figure 2. Illustration of block net set-up.
Upper panel shows the general appearance and orientation of a secure block net. Lower panel emphasizes key steps to secure a block net. Please click here to view a larger version of this figure.



This page demonstrates the ataxic size spectra method applied to macroinvertebrate and fish data collected in a West Virginia stream.

The first step of the size spectra method is to sort individual organisms into log2 size bins, by individual dry mass (mg). Next, the number of individuals in each log2 size bin is summed and used to estimate total density for each size bin (number per square-meter).

Two important data manipulations steps are then taken. First, the density estimates are 'normalized' by dividing the density estimate for each log2 size bin by the width of the respective bin. Next, the body mass (x-axis) and density (y-axis) data are log10 transformed. By using the toggles below, you can see how each of these steps affects a body mass vs. density scatterplot (with ordinary least squares linear model shown) and histogram of summed biomass within each log2 size bin.

Select:

○ Raw data, without normalization or log10 transformation
○ Normalized density, without log10 transformation
○ Log10 transformed mass and density, without normalization
● Normalized density, with log10 transformation

Questions or comments? Please send an email to Daniel McGarvey (djmcgarvey@vcu.edu).

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. Please click here to view a larger version of this figure.

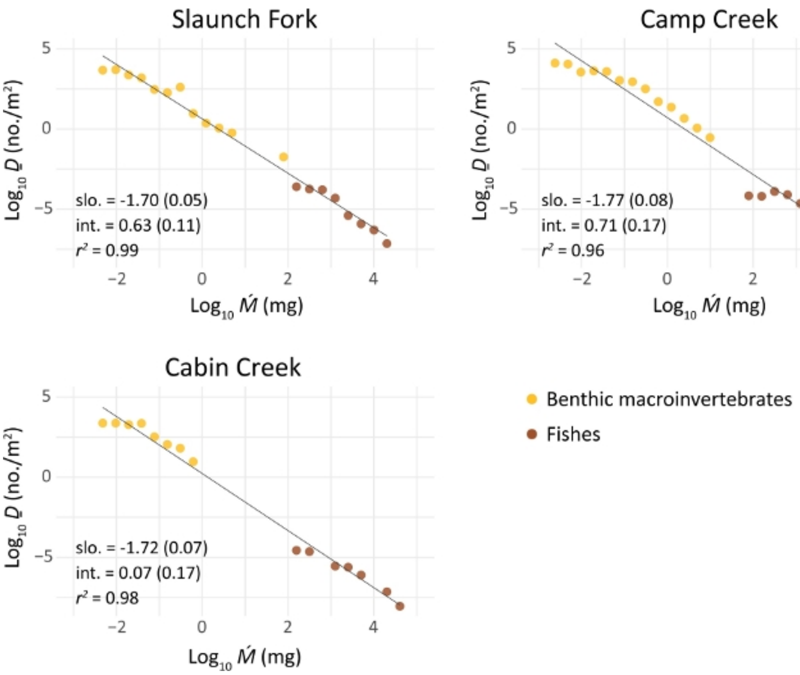


Figure 4. Ataxic size spectra plots from three West Virginia streams.
Benthic macroinvertebrate and fish data are distinguished by color. In each plot, average individual dry mass (*Ḿ*) within $\log_2$ size bins is shown on the *x*-axis and normalized density (*D̲*) is shown on the *y*-axis. Least-squares 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. Please click here to view a larger version of this figure.

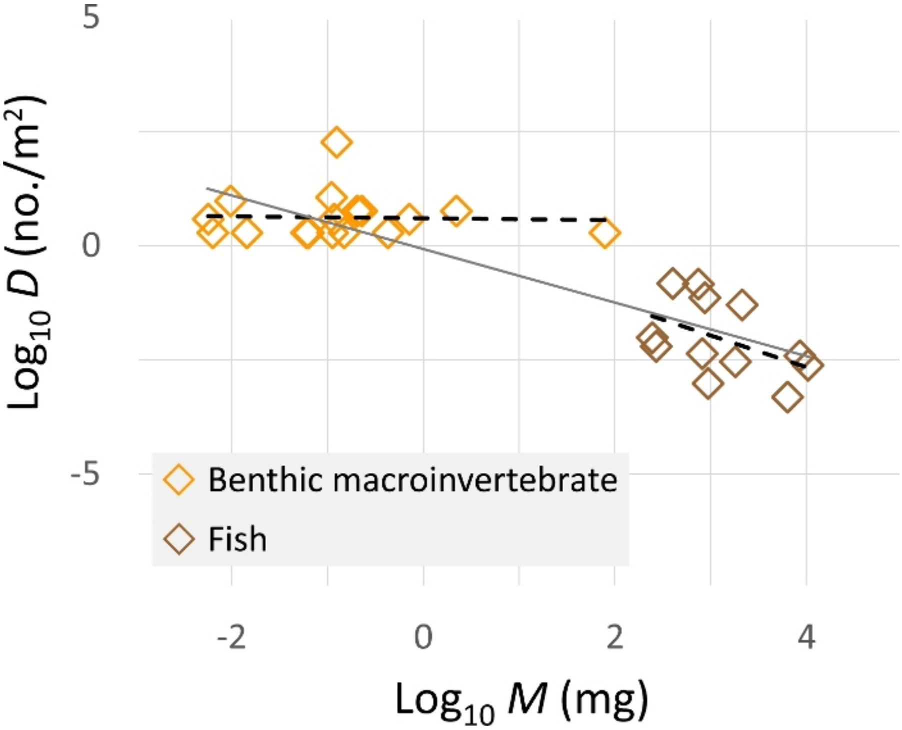


Figure 5. Taxic body mass vs. density relationship in Slaunch Fork.
Each data point (diamond) represents the mean body mass (*M*, dry mass) and estimated density (*D*) of a single taxon. Linear regression models are shown separately for invertebrates and for fishes (dashed black lines), as well as combined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

Supplementary File 1. Example field data sheet used to record fish identities, lengths, and weights. Please click here to download this file.

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. Please click here to download this file.

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[48,49,50], lakes[51,52,53], and marine environments[54,55,56].

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[63,64,65]. 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.

Disclosures

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