



Allometric estimation of earthworm ash-free dry mass from diameters and lengths of select megascolecid and lumbricid species

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ABSTRACT

We present novel length to ash-free dry mass and preclitellar diameter to ash-free dry mass allometric equations for seven earthworm species from the families Megascolecidae and Lumbricidae, all of which are exotic and most of which are of ecological concern in North America: *Eisenia hortensis*, *Eisenia fetida*, *Dendrodrilus rubidus*, *Lumbricus rubellus*, *Octolasion* sp., *Amyntas hilgendorfi*, and *Perionyx excavatus*. We also present a length–biomass allometric equation for one enchytraeid, *Mesenchytraeus* sp. All relationships between length and biomass, and diameter and biomass were statistically significant at the species and family level ($P < 0.001$). The predictive powers of these allometric regressions (as coefficients of determination, r^2) were species-specific, and ranged widely from 0.27 to 0.93. Length–biomass regressions provided more predictive power and precision overall than preclitellar diameter–biomass calculations at both the species and the family levels. An ANCOVA followed by orthogonal contrasts determined that, while the slopes of these regressions did not differ significantly between the two earthworm families, significant differences in slopes of length–biomass regressions existed among species within families, indicating the utility of having species-level equations for accurate biomass predictions. With these allometric relationships, we aim to improve the estimation of earthworm biomass in order to facilitate investigations of how exotic-invasive earthworm species impact soil ecosystems.

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Introduction

Determining the biomass of organisms is a fundamental component of many ecological studies, especially those dealing with growth rates, production, or the influence of organisms on important ecosystem processes such as resource acquisition, decomposition and nutrient cycling. For studies that involve soft-bodied invertebrates, biomass determination can be complicated by the fact that individual wet mass varies with environmental conditions such as humidity. To bypass this problem, researchers frequently measure some aspect of organism size (e.g., length and head-capsule width) and then employ allometric relationships to equate these measurements with dry mass (Benke et al. 1999). Dry mass, or ash-free dry mass (AFDM), provides a measure of biomass that is not influenced by moisture content and is preferred as an expression of biomass over wet mass for many organisms (Bohlen et al. 2004). A key advantage of using allometric equations to estimate dry mass is that it circumvents the need to dry individuals before weighing (Hale et al. 2004), thus preserving the organism's anatomical and chemical integrity for such purposes as cataloging voucher specimens or obtaining samples for genetic analysis.

For earthworms and other invertebrates, species can differ in body size and proportion (Hale et al. 2004), and species-specific allometric equations tend to more accurately predict species biomass than do allometric equations for coarser taxonomic resolutions (Benke et al. 1999). Length to AFDM allometric relationships have been established by Hale et al. (2004) for some common invasive European lumbricids in North America, which have been successfully employed in ecological studies (e.g., Holdsworth et al. 2007; Costello and Lamberti 2008). However, allometric relationships for other common and ecologically important earthworm taxa are lacking.

Increasingly, earthworms of European and Asian origins are invading terrestrial ecosystems in North America, often with undesired ecological impacts (Burtelow et al. 1998; Hale et al. 2005). The genus *Amyntas*, of Asian origin (Lee 1985), is distributed throughout the eastern and southeastern United States, and locally in the Midwest United States (Burtelow et al. 1998; Callahan et al. 2003; Reynolds and Wetzel 2008). Members of the genus *Perionyx*, originating from India (Julka et al. 2009), are common contaminants of earthworm cultures in the vermicomposting industry in North America (Greiner and Tiegs, personal observation). Each of these genera belongs to the family Megascolecidae, which is characterized by a high potential for colonization and invasion (Brown et al. 2006). As of yet, allometric equations have not been developed for this family.

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Similarly, while at least 25 species of the family Lumbricidae have become established in North America (Hendrix and Bohlen 2002), allometric relationships have been developed for only a small subset.

Here we present allometric equations relating length to biomass for seven earthworm species from the families Megascolecidae and Lumbricidae. Additionally, we relate preclitellar diameter to biomass, an approach that offers methodological advantages in some instances. For example, Jimenez et al. (2000) found that earthworm diameter was a reliable predictor of biomass for earthworms collected in Colombia, and the technique was useful when specimens were damaged during the collection process and the entire earthworm was not available. We also developed a length–AFDM allometric equation for the microdrile, *Mesenchytraeus* sp., which is a giant enchytraeid similar to earthworms in ecological function. This is the first known allometric relationship developed for this taxonomic group. With these allometric equations, it is our goal to facilitate investigations of invasive earthworms in order to better understand their impacts on terrestrial ecosystems.

Materials and Methods

Amyntas hilgendorfi individuals were collected by hand from riparian soils in southeastern Michigan and identified using the key of Reynolds (1978). Three commonly used composting earthworms – *Eisenia fetida*, *Eisenia hortensis*, and *Perionyx excavatus* – were acquired from vermicomposting suppliers and identified according to Blakemore (2002). *Lumbricus rubellus*, *Octolasion* sp. (probably *O. tyrtaeum*), and *Dendrodrilus rubidus* were collected by hand from riparian soils on Prince of Wales Island, Southeast Alaska and identified using Reynolds' key (1977). *Mesenchytraeus* sp. was also collected from Prince of Wales Island and identified according to Dindal (1990). Since juvenile *L. rubellus* are not distinguishable from the congeneric, *L. terrestris* (Hale et al. 2005), juvenile *L. rubellus* were taken from a pure laboratory culture and used to supplement the data obtained from adults collected in the field. All specimens were preserved in 70% ethanol for a minimum of four days before size measurements were taken.

Lengths of individual specimens were determined by aligning them along the edge of a ruler and measuring the total length to the nearest 0.5 mm three separate times. For clitellated individuals, the diameter of the first segment anterior to the clitellum was measured with digital calipers to the nearest 0.01 mm three separate times. Since *Mesenchytraeus* sp. lacked a distinguishable clitellum, diameter measurements were not made for this group. Means of the three length and diameter measurements were used in analyses. After length and diameter determination, specimens were placed in a drying oven for a minimum of 24 h at approximately at 60 °C, and then cooled in a desiccator for a minimum of 1 h prior to measuring dry mass (± 0.1 mg). Samples were then ashed at 500 °C for a minimum of 4 h. The remaining ash was weighed (± 0.1 mg) and the value was subtracted from dry mass to determine the AFDM for each individual earthworm. Small individuals with an initial dry mass less than 10 mg were pooled with others of a similar length to calculate a mean AFDM (after Hale et al. 2004). Each set of pooled individuals constituted a single data point on allometric regressions, using the average length of the combined specimens.

Statistical Analysis

Ordinary least-square regression was performed on natural-log-transformed data to develop linear relationships between

length and AFDM as well as preclitellar diameter and AFDM for each species measured. These regressions provided values for a and b of the function: $\ln[\text{AFDM}] = b \cdot \ln[\text{length or diameter}] + a$. The AFDM of an individual *Octolasion* sp. was identified as an outlier (studentized residual > 2.0) and was excluded from analyses. Regressions were also developed for length–AFDM and preclitellar diameter–AFDM relationships for the families Lumbricidae and Megascolecidae by combining data gathered for their respective species. To ensure all species were equally influential in the family-level equations, a number of individuals were randomly selected from each species equal to the sample size of the species with the least number of individuals for that family. Percent errors for species- and family-level equations were calculated for each specimen measured using the formula $|\text{predicted AFDM} - \text{actual AFDM}| / \text{actual AFDM}$. Analysis of covariance (ANCOVA) was used to test for differences in the slopes of regression lines among earthworm taxa. Following the ANCOVA, orthogonal contrasts were employed to determine if the morphometric differences described by our allometric equations could be predicted by taxonomy. The slopes were compared first among families, then among genera and lastly, species.

Results

Slopes of the regression lines used to predict earthworm biomass from length differed significantly among species ($F_{7,796} = 28.3$, $P < 0.001$). Orthogonal contrasts revealed no difference in slopes between the families Megascolecidae and Lumbricidae ($P = 0.53$). The slope of the length–biomass regression for the single enchytraeid species we examined was significantly different from that of megascolecid and lumbricid families ($P < 0.001$). Within the family Megascolecidae, *P. excavatus* and *A. hilgendorfi* had significantly different slopes ($P < 0.001$). Within the family Lumbricidae, the slope for *L. rubellus* was different from all others ($P < 0.001$) and slopes for *D. rubidus* and *O. cyaneum* were significantly different ($P = 0.02$) from those for *E. hortensis* and *E. fetida*.

The slopes of preclitellar diameter–AFDM regression lines differed significantly among species ($F_{6,564} = 7.68$, $P < 0.001$). Slopes of regression lines for the families Megascolecidae and Lumbricidae regressions were marginally different ($P = 0.05$). At the species level with the family Megascolecidae, the slope for *P. excavatus* was significantly different from that of *A. hilgendorfi* ($P < 0.001$). Within the family Lumbricidae only the slope for *L. rubellus* was significantly different from those of the four other lumbricid species ($P < 0.001$).

Percent error (i.e. the difference between actual and estimated values) was consistently greater for regressions at the family level than at the species level for both length and diameter measurements. When species-level regressions were used rather than family-level equations, biomass estimation for *A. hilgendorfi* improved by 7.1%; *P. excavatus* improved by 26.1%; *L. rubellus* improved by 6.8%; *E. fetida* improved by 22.3%; *E. hortensis* improved by 0.4%; *D. rubidus* improved by 10.7%; and *Octolasion* sp. decreased by 23.1%. Similarly, mean diameter–AFDM estimation improved by 0.4% for *A. hilgendorfi*, 21.5% for *P. excavatus*, 3.9% for *L. rubellus*, 3.5% for *E. fetida*, 3.4% for *E. hortensis*, 23.0% for *D. rubidus*, and 0.1% for *Octolasion* sp.

Length–AFDM and preclitellar diameter–AFDM regressions were statistically significant ($P < 0.001$) across all species and predictive powers (i.e., r^2) were species-specific (Figs. 1 and 2). Information on mean lengths and diameter, size range, and sample size is provided in Table 1 for each species. Overall, diameter regressions provided less predictive power than the length–AFDM relationships. The diameter–AFDM regression

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