

A semi-automated digital microphotographic approach to measure meiofaunal biomass

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Abstract

Meiofauna studies often investigate community structure and function where biomass estimates and taxonomic analysis are required. Nondestructive methods of biomass estimation are necessary to preserve specimens for subsequent taxonomic analysis. A semi-automated protocol to estimate meiofaunal biomass was developed to meet this need. The method improves upon previous indirect biomass techniques by using digital microphotography and analytical graphics software to obtain better estimates of biovolume. The technique is not fully automated because the digital images require some manipulation. Dry mass and carbon mass were estimated for two dominant components of marine benthic meiofauna (Nematoda and Harpacticoida) as the product of conversion factors and body volumes. The technique was validated by comparing indirect dry and carbon mass estimates to direct measurements using an analytical balance and carbon-hydrogen-nitrogen (CHN) elemental analyzer. No significant difference was found between the semi-automated method and direct measurements for harpacticoid dry or carbon mass ($P = 0.68$ and $P = 0.74$, respectively), or nematode dry mass ($P = 0.28$) or carbon content ($P = 0.17$). The semi-automated indirect method was used to estimate the biomass of meiofauna (13,279 harpacticoids and 12,288 nematodes) collected from the deep-sea Gulf of Mexico. Estimated average wet mass was compared to direct analytical balance measurements from an earlier, independent study in the same area. Wet mass estimates generated by the indirect method ($2.67 \pm 0.86 \mu\text{g/harpacticoid}$ and $0.85 \pm 4.78 \mu\text{g/nematode}$) were similar to direct measurements in the earlier study ($2.80 \mu\text{g/harpacticoid}$ and $0.85 \mu\text{g/nematode}$). The semi-automated indirect method is about three times faster than traditional microscope methods to measure body volume, estimates biomass comparably to direct methods, and conserves samples and images of samples for other analyses.

Measures of standing stocks (i.e., biomass) of ecosystem components are required to understand ecological energetics in terms of individual organisms or entire ecosystems. Community structure identification relies upon taxonomic analysis and multivariate determinations of community differences between areas (Warwick and Clarke 1991; Montagna and Harper 1996). Therefore, meiofauna studies often require non-destructive methods for biomass estimation to preserve specimens for subsequent taxonomic analysis.

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In marine sediments, meiofauna play an important role in mediating microbial processes and cycling of organic carbon (Montagna 1995). Meiobenthic samples typically are dominated by two taxa: Harpacticoida and Nematoda (Higgins and Thiel 1988; Giere 1993). Nematodes generally dominate the sample contributing 70% to 95% of total individuals while harpacticoids constitute a lesser proportion ranging from 5% to 20%. Other taxa usually comprise a minor proportion of individuals ranging from 5% to 20%. For this reason, meiofaunal biomass is frequently based on harpacticoid and nematode values alone (Montagna 2002). Numerous techniques have been devised for measuring meiofaunal biomass. Small size, high abundance, and taxonomic complexity of meiofaunal communities make biomass estimations difficult and labor intensive.

Biomass may be expressed in terms of wet mass, dry mass, ash-free dry mass, carbon mass, or nitrogen mass (Feller and Warwick 1988; Giere 1993; Soltwedel 2000). Many techniques are used to estimate these values, but each can be categorized as a direct or indirect approach. Direct methods rely on electronic instruments (e.g., analytical balance or elemental ana-

lyzer) to measure sample pools (i.e., an aggregate of individuals whose number is determined by minimum sensitivity of the instrument). Indirect estimates of meiofaunal biomass resemble methods used to quantify microbial biomass (Kemp et al. 1993). These methods produce estimates based on a combination of independent factors including counts, biovolume determined from measures of individual body size (length and width), conversion factors determined from body form analysis and chemical measurements, and assumptions regarding specific gravity and density of organisms. Body volumes can be estimated using a variety of methods including hand drawings produced using a camera lucida and calibrated ocular micrometers mounted on compound or dissection microscopes, photomicrographs, or video imaging systems (Feller and Warwick 1988; Montagna 2002). Biovolume estimates and conversion factors required by indirect methods increase opportunities for error, because error associated with multiple independent factors can be propagated at each stage of calculation. However, indirect methods can be performed on individuals, allowing population studies and preserving samples for taxonomy. In contrast, direct methods require sample aggregation and destruction.

Improvements to the indirect measurement technique would include reduction in labor-intensive length and width measurements, increased accuracy from improved conversion factors, and verification of the many assumptions. In this report, conversion factors used in previous studies were reviewed, and carbon-to-dry mass conversion factors were measured empirically. A semi-automated method is presented for estimating biovolume of Harpacticoida and Nematoda. This semi-automated method improves upon previous indirect techniques by taking advantage of digital microphotography and analytical graphics software to reduce the effort needed to make measurements while increasing the accuracy of biovolume estimates. The method was applied to benthic meiofaunal samples collected on the continental slope and rise of the northern Gulf of Mexico.

Materials and procedures

Field methods—Sediment samples were collected using 5.5 cm (inner diameter) subcores mounted inside a GOMEX box core (Boland and Rowe 1991). Samples were taken at depths ranging from approximately 200 m to 3200 m on the continental slope, rise, and abyssal plain in the northern Gulf of Mexico (north of 26°N) from the Florida Escarpment to the Texas-Mexico border. Cores were extruded to the 3 cm sediment depth horizon and placed in 500-mL plastic jars. To prevent undesirable shrinkage or contraction of soft-bodied animals, 50 mL MgCl₂ (7%, isotonic to seawater) was added to each sample. Samples were preserved with enough formalin (formaldehyde diluted to 10% with isotonic 16 μm sieved seawater buffered with borax) to approximate a 4% solution. Samples were returned to the lab where they were stored for a minimum of one and maximum of twelve months prior to processing.

Animal extraction—Samples were sieved initially over 45 μm mesh to remove bulk silt and clay. Meiofauna were extracted from sediment using isopycnic centrifugation in Ludox-AM® silica sol, as described by Burgess (2001). Nematodes and harpacticoids were removed from total meiofauna using a dissecting microscope, and taxa were placed separately in glycerol droplets on 2- × 3-inch glass ring slides in preparation for digital photography.

Digital photography—All harpacticoids and subsamples of 30 nematodes per sample were photographed with a Kodak DC290 Zoom Digital Camera mounted on a Zeiss compound light microscope fitted with a Plan 2.5 10/8 objective (Fig. 1A, 1B). Animals were placed in a droplet of glycerol on a glass slide. The number of harpacticoids included per photograph ranged from 1 to more than 25, depending on the size of individuals. Generally, no more than 15 nematodes were included per photograph. Care was taken not to overlap animals to ensure discrete data capture per individual. Otherwise, no attempt was made to alter the orientation of individuals. This was not a problem with nematodes because of their simple body shapes and general lack of appendages. Most settled out in the glycerol medium and lay flat against the glass slide. However, harpacticoids, because of their relatively complex morphology, and morphological differences between families and genera, frequently remained suspended in the glycerol at variable angles to the photographic plane. This problem was solved by applying a correction factor of 1.5 (see *Biomass calculation*) for variable orientation, as an alternative to spending excessive time manipulating individual animals. The convex upper surface of glycerol drops did not distort images for two reasons: (1) the glycerol drop was allowed to settle before photography so that it became very thin and flat, and (2) the area of focus was sufficiently small that it approximated a flat surface.

Remote camera operation was controlled via a Kodak MDS 290 software interface with a Dell P780 PC. Default camera settings were maintained with the following exceptions: "Capture Settings" were set for "manual infinity focus" and "tungsten white balance." "Picture Type" options were set for "best picture quality" and resolution was set at "medium" level (1440 × 960 pixels) to match the PC screen resolution (1280 × 1024 pixels). "Picture Capture" zoom was consistently maintained at "50 mm." Area and distance was calibrated from a photograph of a 2.0-mm micrometer.

Data capture—SigmaScan Pro 5 was used to calculate physical measurements (approximate two-dimensional area and mid-body width of photographic images) of individual animals in units of micrometers. Microsoft Excel worksheets generated by SigmaScan were produced following a multi-step process. Area and distance were calibrated in micrometer units using the "two-point calibration" option with a photograph of a 2.0-mm micrometer. Three discrete color "overlays" were applied to each animal for numeric identification and measurement purposes (Fig. 1C, 1D). The first overlay

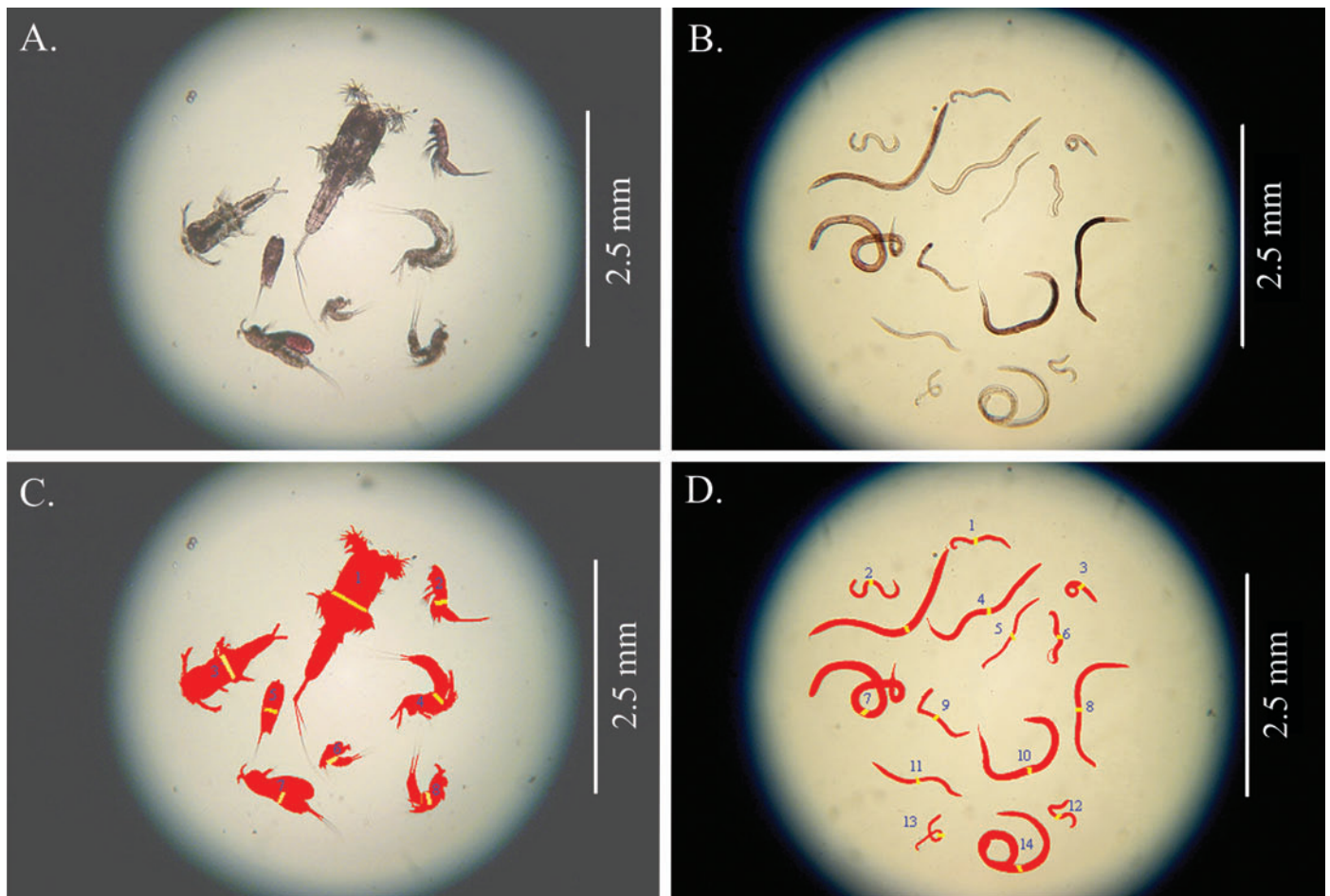


Fig. 1. Photomicrographs of harpacticoids (A) and nematodes (B) using a Kodak DC290 digital camera (set at a medium resolution of 1440×960 pixels) mounted on a Zeiss compound light microscope fitted with a Plan 2.5 10/8 objective. Camera operation was controlled via Kodak MDS 290 software interface and Dell P780 PC. Photomicrographs of harpacticoids (C) and nematodes (D) showing application of three discrete color overlays per animal for numeric identification and measurement by SigmaScan software. A blue overlay assigned unique numeric identifiers to each animal. A red overlay was used to approximate area of individual images. A yellow overlay was used to approximate mid-body width of each animal.

required “Fill” and “Draw” tools to approximate the area of each photographic image. A second overlay assigned unique numeric identifiers to each animal by selecting the “Measure Objects” command. This overlay was turned off during subsequent use of the “Measure Objects” command to avoid multiple numeric assignments. The third overlay approximated mid-body width of each animal using the “Distance” tool to measure individuals in the previously defined numerical sequence. SigmaScan produced Excel data columns for area and width independently after selecting overlay type (i.e., fill or mid-body line), appropriate measurement type (i.e., “Area” or Distance”), and the “Measure Objects” command. A flow chart of the SigmaScan software procedures is presented in Fig. 2.

Biomass calculation—The area (A) and width (W) generated by SigmaScan were used to convert raw data into biomass estimates. Nematode biovolume (V, in nL units) esti-

mates were calculated from two separate equations to determine which method was most accurate in comparison to direct biomass measurements. The first equation used to determine nematode biovolume is based on the volume of a rectangular cube, but corrects for circular body shape by applying a single conversion factor ($C_{bf} = 530$) determined by Warwick and Price (1979):

$$V \text{ (nL)} = [A \text{ (mm)} \times W \text{ (mm)}](C_{bf})/10^9. \quad (1)$$

The conversion factor (C_{bf}) accounts for two estimated body volume components: (1) the hemispherical head section of each nematode and (2) 16 equally spaced points along the length of each nematode and assuming circular cross-sections (Warwick and Price 1979). In contrast, the authors suggest using a second method of nematode biovolume estima-

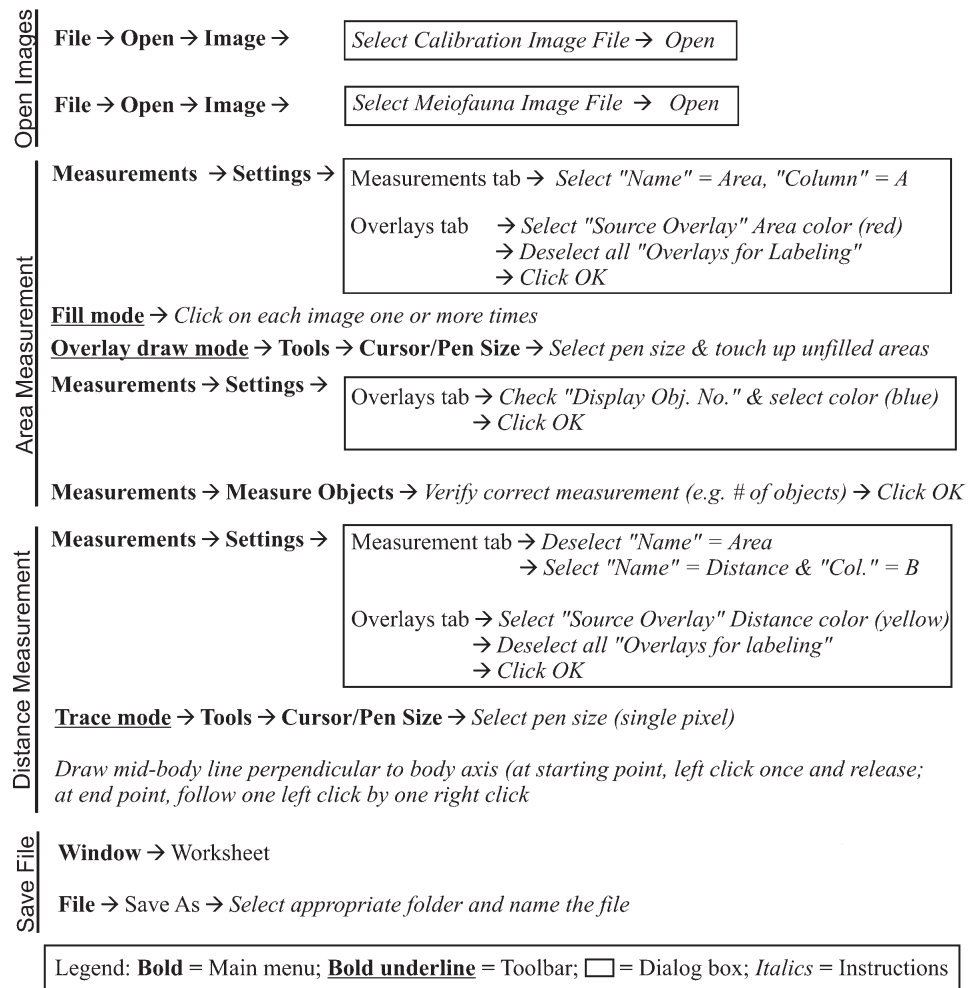


Fig. 2. Detailed instructions of SigmaScan software manipulation. Flow chart includes each step involved in measuring meiofauna body volume from a digital microphotograph. The flow chart legend differentiates main menus, toolbars, dialog boxes, and literal instructions.

tion by assuming the volume of a cylinder:

$$V \text{ (nL)} = \pi r^2 L / 10^6 \quad (2)$$

where L equals the total length of the nematode (area/width) and r equals the radius (mid-body width/2).

Harpacticoid biovolume calculation relied on area and width measurements along with two conversion factors (C_{bf} = body form and C_o = orientation). Body volume was estimated from a formula used by Feller and Warwick (1988) and Warwick and Price (1979) to measure harpacticoid biovolume:

$$V \text{ (nL)} = [A \text{ (mm)} \times W \text{ (mm)}] (C_{bf} \times C_o) / 10^9. \quad (3)$$

Harpacticoid body volume estimates relied on eight body

type-specific conversion factors (C_{bf}) derived from volumetric displacement of plasticene scale models (McIntyre and Warwick 1984; Warwick and Gee 1984). Application of these factors required matching SigmaScan images of individual harpacticoids to line drawings of different body forms (cylindrical, semi-cylindrical compressed, semicylindrical, semicylindrical depressed, fusiform, pyriform, pyriform depressed, and scutelliform) and their corresponding conversion factors (Table 1). Photographic images that did not approximate one of these eight body forms because of variable axial orientation and rotation were assigned a default value ($C_{bf} = 440$). The default value was derived from average conversion factors of five of the most commonly encountered body forms (semicylindrical, semi-cylindrical depressed, fusiform, pyriform, pyriform depressed). One additional conversion factor was required to account for the average loss of image area resulting from variable body ori-

Table 1. Harpacticoida body forms and corresponding conversion factors (Warwick and Gee 1984)

| Body Forms | Conversion factor (C) |
|----------------------------|-----------------------|
| Cylindrical | 750 |
| Semicylindrical compressed | 630 |
| Semicylindrical | 560 |
| Semicylindrical depressed | 490 |
| Fusiform | 485 |
| Pyriiform | 400 |
| Pyriiform depressed | 260 |
| Scutelliform | 230 |

entations. The longitudinal axis of most animals was generally parallel (~0°) to the photographic plane displaying a ventral, dorsal, or lateral aspect, as desired. However, individuals were frequently oriented at an angle to the photographic plane (1° to 90°), yielding underestimates of biovolume. A correction factor ($C_o = 1.5$) was determined by comparing the average biovolume of 90 harpacticoids photographed in the standard mode (average biovolume = 1.17 ± 1.09 nL) to the biovolume of the same individuals after being manipulated into a flat, noncompressed, dorsal orientation (average biovolume = 1.75 ± 2.19 nL).

Nematode and harpacticoid wet mass was calculated from biovolume using a specific gravity of 1.13, and wet mass was converted to dry mass assuming a ratio of 25% (Weiser 1960;

Feller and Warwick 1988). Previous studies have used a carbon-to-dry mass ratio of 40% (Feller and Warwick 1988; Warwick and Price 1979; Danovaro et al. 1995; and others), which was estimated for chaetognaths by Steele (1974). We found carbon to dry mass ratios of 51.4% for nematodes and 45.8% for harpacticoids by direct measurement (Table 2). We used these empirical values to convert dry mass to carbon mass.

Direct biomass measurements—Comparative measurements of dry mass and carbon content were used to validate the semi-automated indirect method. Cultured harpacticoids were used for direct biomass measurement. Large harpacticoids of a consistent size range were desired to minimize the number of individuals per sample pool, but still exceed the minimum sensitivity requirements of the analytical instruments. Because wild harpacticoids satisfying these characteristics were not readily available in sufficient numbers, cultured adult harpacticoids (*Amphiascoides atopus*, from Dr. John Fleeger, Louisiana State University) were used. Mean two-dimensional dorsal surface area of these animals was $148,326 \mu\text{m}^2 \pm 39,140 \mu\text{m}^2$. Mean mid-body width was $177 \mu\text{m} \pm 26 \mu\text{m}$. Extrapolated mean body length was $838 \mu\text{m}$. Specimens were preserved in 10% formalin, frozen prior to shipment, and processed one to three weeks after receipt. Freezing of preserved harpacticoid samples was assumed to not alter biomass. However, direct loss in both dry mass and carbon content is known to result from formalin preservation (Feller and Warwick 1988). In the case of zooplankton, most of this loss occurs during the first 7 d

Table 2. Literature values of experimentally determined conversion factors required to calculate meiofaunal biomass by the semi-automated approach

| Reference | W/D* | C/D* | C/W* | SG* |
|--------------------------------|---------------|---------------|---------------|---------------|
| Nematoda | | | | |
| Overgaard Nielsen† (1949) | | | | 1.02 |
| Bair† (1955) | | | | 1.02 |
| Andrassy‡ (1956) | | | | 1.084 |
| Weiser§ (1960) | 0.25 | | | 1.13 |
| Silvapalan and Jenkins† (1966) | 0.208 | | | |
| Buecher and Hansen† (1971) | 0.2 | | | |
| Montagna§ (1983) | | 0.403 | | |
| Jensen§ (1984) | | | 0.124 ± 0.013 | |
| This study§ | | 0.514 | | |
| Copepoda | | | | |
| Omori (1969) | 0.203 | 0.579 | | |
| Durban and Durban (1978) | | 0.473 | | |
| Ikeda and Mitchell (1982) | | 0.433 | | |
| Montagna§ (1983) | | 0.303 | | |
| This study§ | | 0.458 | | |
| Mean ± SD | 0.215 ± 0.023 | 0.452 ± 0.087 | 0.124 ± 0.013 | 1.064 ± 0.054 |

*W/D, wet/dry mass; C/D, carbon/dry mass; C/W, carbon/wet mass; and SG, specific gravity.

†Terrestrial.

‡Freshwater.

§Marine.

||Planktonic marine.

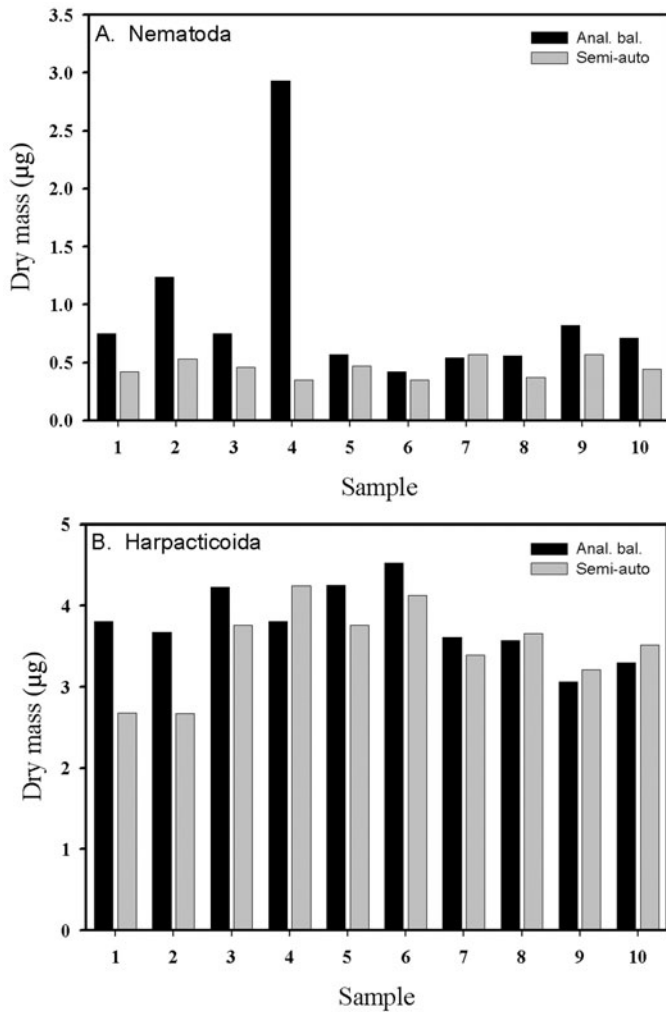


Fig. 3. Average dry mass per individual nematode (A) and harpacticoid (B) samples (100 individuals/sample pool) estimated by the semi-automated method and measured by analytical balance.

following preservation with a trend toward stabilization after approximately 30 d (Durbin and Durbin 1978; Omori and Ikeda 1984; Widbom 1984). Loss of biomass due to leaching or other preservation artifacts was corrected by applying conversion factors of 1.26 (i.e., 26%) for dry mass and 1.14 (i.e., 14%) for carbon content (derived from Durbin and Durbin 1978).

Direct biomass measurements of nematodes were carried out using animals collected from the marina at the University of Texas Marine Science Institute. Sediment cores from a water depth of ~3 m were collected with a 5-cm diameter coring tube attached to a PVC pole. Surface sediments (~upper 2 cm) were retained for extraction using the isopycnic protocol described above with one modification: samples were not preserved (e.g., formalin and ethanol) prior to processing to avoid the negative effect (i.e., loss of dry mass and carbon content) on direct measurements (Durbin and Durbin 1978; Omori and Ikeda 1984). Instead, samples were processed immediately or

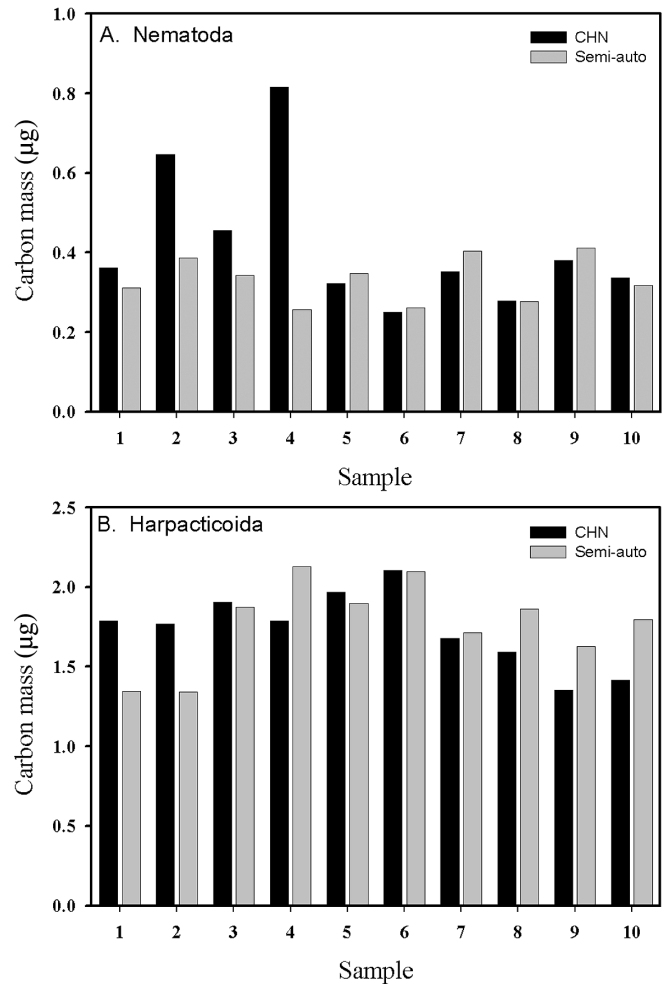


Fig. 4. Average carbon mass per individual nematode (A) and harpacticoid (B) samples (100 individuals/sample pool) estimated by the semi-automated method versus direct measurement by CHN analyzer.

stored in a refrigerator at 2°C for a maximum of 3 d prior to processing. Freshwater was used during the extraction process to avoid trapping specimens in an insoluble matrix of silica crystals formed by reaction between divalent cations in seawater during isopycnic extraction (Burgess 2001). Exposure of living or moribund nematodes to freshwater during the sieving process caused osmotic damage in some individuals. The nematode cuticle is very rigid and impermeable, disallowing body swelling with decreased external osmotic pressure. However, osmotic damage can result in extrusion of internal structures through the anus or vaginal aperture. Obviously affected individuals were not selected for measurement to prevent over-estimation of two-dimensional body areas from digital photographs. Nematode individuals of similar size were selected for measurement. Mean two-dimensional dorsal surface area of these animals was $51,989 \mu\text{m}^2 \pm 19,526 \mu\text{m}^2$. Mean mid-body width was $56 \mu\text{m} \pm 11 \mu\text{m}$. Extrapolated mean body length was 928 μm .

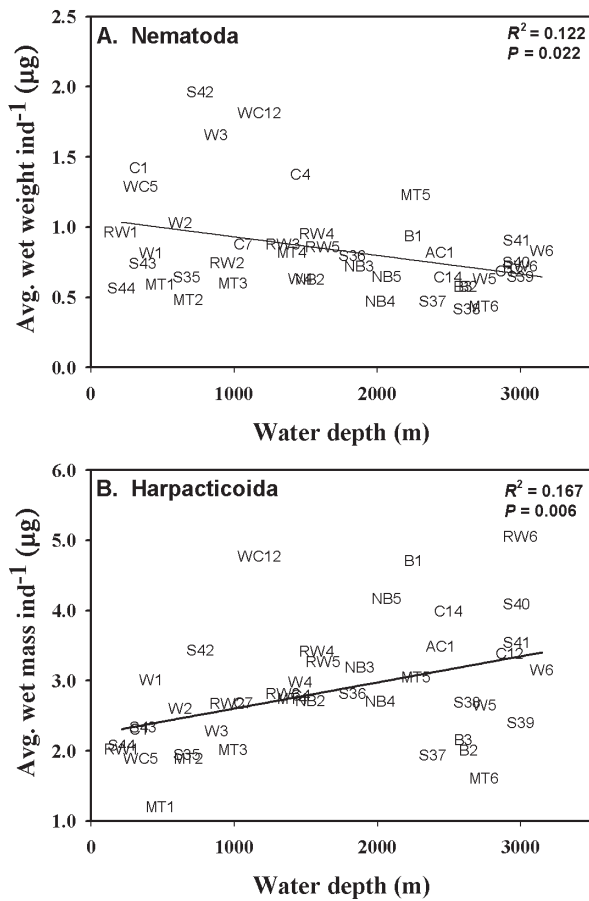


Fig. 5. Average wet mass per individual nematode (A) and harpacticoid (B) determined by the semi-automated method for 43 northern Gulf of Mexico stations. A solid line determined by linear regression indicates decreasing weight with increasing depth for nematodes opposed to a trend of increasing weight with increasing depth for harpacticoids.

Dry mass measurements were made using a Mettler Toledo MT5 analytical balance (sensitivity of $\pm 1 \mu\text{g}$). Specimens were collected and placed on small tin weighing boats, dried to a constant weight (at 60°C for 24 h), and stored in a desiccator prior to weighing (Feller and Warwick 1988; Montagna 2002). Direct measures of meiofauna carbon content were made using a Carlo Erba EA1108 Elemental Analyzer (detection limit of 10 ppm, range of 0.1 to 100 mg (solid samples), accuracy of $<0.3\%$ absolute, and repeatability of $<0.2\%$ absolute). Ten harpacticoid and 10 nematode sample pools, each containing 100 individuals, were measured by the three methods (i.e., semi-automated, analytical balance, and elemental analyzer). Sample sizes exceeded minimum sensitivity of test equipment by at least 50%.

Statistical analysis—Differences between the semi-automated method and mass measurement using analytical balance and elemental (CHN) analyzer were tested using a paired *t* test with SAS statistical software, version 8.2 (SAS Institute Inc. 1991).

Assessment

Indirect versus direct methods comparison—Nematode indirect dry mass estimates by Eq. 1 were nearly statistically different from direct measurements ($P = 0.07$) and were lower than direct measurements in 9 of 10 replicates. Mean (\pm standard deviation) dry mass of individual nematodes using Eq. 1 was $0.44 (\pm 0.07) \mu\text{g}$, compared to a mean of $0.929 (\pm 0.74) \mu\text{g}$ using an analytical balance. Nematode dry mass estimated using Eq. 2 was not significantly different than direct measurements ($P = 0.28$, Fig. 3A). Mean nematode dry mass using Eq. 2 was $0.65 (\pm 0.11) \mu\text{g}$, compared to a mean of $0.93 (\pm 0.74) \mu\text{g}$ using an analytical balance. The higher analytical balance mean was strongly affected by a single unusually high value for sample number 4 (Fig. 3A). If sample 4 is removed from the data set, mean nematode dry mass by direct measurement becomes $0.71 \mu\text{g}$, which is very close to the mean nematode dry mass ($0.65 \mu\text{g}$) estimated by Eq. 2 ($P = 0.30$).

No significant difference was found for semi-automated indirect estimates compared to direct measurements of harpacticoid dry mass ($P = 0.68$, Fig. 3B). Mean (\pm standard deviation) harpacticoid dry mass using the semi-automated indirect method was $3.86 (\pm 0.56) \mu\text{g}$, compared to a mean of $3.78 (\pm 0.43) \mu\text{g}$ for direct measurements.

Nematode carbon estimates obtained using Eq. 1 were significantly different from direct measurement by CHN analysis ($P < 0.01$, Fig. 4A; Eq.1 mean \pm SD = $0.22 \pm 0.04 \mu\text{g}$; CHN analysis mean \pm SD = $0.42 \pm 0.18 \mu\text{g}$). No significant difference between the semi-automated indirect method and the direct method was found for nematode carbon mass when Eq. 2 was used ($P = 0.17$, Fig. 4A). Mean carbon content per nematode using Eq. 2 was $0.33 (\pm 0.06) \mu\text{g}$ compared to a mean of $0.42 (\pm 0.18) \mu\text{g}$ by CHN analysis. Nematode carbon content obtained by the indirect method was lower than direct measurements in all 10 samples using Eq. 1, but was lower in only 6 of 10 samples using Eq. 2.

No significant difference was found between carbon mass measurements of harpacticoid samples using the semi-automated indirect method compared to CHN analysis ($P = 0.74$, Fig. 4B). Mean (\pm standard deviation) carbon content per individual harpacticoid using the semi-automated method was $1.77 (\pm 0.26) \mu\text{g}$ compared to a mean of $1.74 (\pm 0.22) \mu\text{g}$ using a CHN analyzer.

The semi-automated method relies on assumptions about the geometric relationship between length and width of individuals and the accuracy of factors to convert body volume to biomass, as well as carbon content. Harpacticoid and nematode biomass estimates using the semi-automated method compared favorably with direct analytical balance and elemental analyzer measurements (Figs. 3 and 4). Nematode biomass obtained from an equation based on previous literature was statistically less than direct measurement (Eq. 1). However, nematode dry and carbon mass was not statistically different from direct measurements when calculated from an equation based on a simple cylindrical body shape (Eq. 2).

The coefficient of variation (CV) for nematode semi-automated biomass estimates indicates greater precision (CV = 16.9% and 18.2% for dry and carbon mass, respectively), than direct measurements by analytical balance or elemental analyzer (CV = 79.6% and 42.9%, respectively). Harpacticoid semi-automated dry and carbon mass estimates had similar precision (CV = 14.5% and 14.7%, respectively) compared to direct analytical balance and CHN measurements (CV = 11.3% and 12.6%, respectively).

Discussion

There is considerable variability in published conversion factors of dry/wet mass, carbon/dry mass, and specific gravity for nematodes and harpacticoids (Table 2). These conversion factors have not been measured for all meiofauna taxa. Previous studies have relied on the uniform application of a single conversion factor (carbon/dry mass = 40%) to determine carbon content (Feller and Warwick 1988; Warwick and Price 1979; Danovaro et al. 1995; and others). This ratio was first estimated by Steele (1974) for chaetognath biomass, but actual data were not presented. The 40% conversion factor did not prove satisfactory for estimation of nematode or harpacticoid biomass in this study. Carbon-to-dry mass ratios were empirically determined for both nematodes (51.4%) and harpacticoids (45.8%) and used for indirect estimation of biomass. More detailed empirical investigation of dry/wet mass and carbon/dry mass ratios is needed. Application of Steele's (1974) 40% ratio may have resulted in consistent underestimation of carbon content by previous investigators.

The semi-automated method compares favorably to previous investigations in the northern Gulf of Mexico. Pequegnat et al. (1990) estimated pooled sample biomass from several stations from the northern Gulf of Mexico deep sea. Average wet mass per individual was calculated by dividing pooled wet mass by the total abundance of each taxon. Conversion factors of 0.85 μg wet mass/individual and 2.8 μg wet mass/individual were calculated for nematodes and harpacticoids, respectively. In the present study of the northern Gulf of Mexico deep sea, comparable conversion factors were calculated for 13,279 harpacticoids and 12,288 nematodes using digitally generated wet mass. Total biomass per taxon was divided by respective abundance values to calculate conversion factors of 0.85 (\pm 4.78) μg wet mass/individual nematode (Eq. 2) and 2.67 (\pm 0.86) μg wet mass/individual harpacticoid (Eq. 3). Average wet mass was identical for nematodes and only 4.6% lower for harpacticoids compared to the pooled averages calculated by Pequegnat et al. (1990). The standard deviation for nematodes is high but reflects the large size range of this taxon in deep Gulf of Mexico sediments. The high standard deviation for nematodes emphasizes the need for indirect measurements of individuals versus pooling of samples for direct measurement because a better understanding of within and between sample variability is generated. Average biomass per individual nematode

was found to decrease with increasing water depth (Fig. 5A; $R^2 = 0.161$), as reported in a previous study (Soltwedel et al. 1996). In contrast, the average biomass per individual harpacticoid generally increases with increasing water depth (Fig. 5B; $R^2 = 0.167$). Thus, higher average biomass would be expected in the current study, because sampling included a greater proportion of stations deeper than 2000 m (19/43 compared to 3/15 sampled by Pequegnat et al. 1990). However, average harpacticoid wet mass per individual in the current study was 4.6% lower than that found by Pequegnat et al. (1990). This may suggest a slight underestimation by the semi-automated method.

Comments and recommendations

The semi-automated method to quantify meiofaunal biomass is an improvement over previous indirect methods. The new method eliminates the tedious and time-consuming effort needed to manually measure the physical dimensions of extremely small organisms. Semi-automated estimates of harpacticoid biomass are comparable to direct measurements, and nematode estimates are not unreasonably lower than direct measurements. We estimate that the semi-automated method of indirect biomass measurement decreases sample processing time by a factor of three in comparison to manual measurement of each (based on hand measurement of 30 nematodes and 30 harpacticoids and manual data entry into a spreadsheet). A second benefit of the semi-automated method is the permanent and easily accessible digital photographic record of each and integrated measurement overlays that are cross-referenced to data sheets.

The semi-automated method can be applied to other meiofaunal taxa using body form conversion factors presented by Feller and Warwick (1988). Soft-bodied meiofauna undergo shrinkage and severe body distortion during bulk fixation. Applying the method to these taxa would require live, and likely narcotized, samples to accurately estimate body volume.

Disadvantages of the semi-automated approach exist. Color overlay of body area is based on contrast between animals and background lighting. Meiofauna, being semi-transparent, often have body parts with very little contrast, even when microscope lighting is adjusted carefully. About 50% of sample images needed manual touch-up to accurately estimate body area. Although touch-up is generally minor and can be done quite rapidly, it decreases automation and increases effort. A skilled technician or graduate student is able to complete a typical deep-sea meiofauna sample of 60 to 90 individuals in 1.5 h. While this is more time consuming than direct measurement with an analytical balance or CHN analyzer, it allows for a sample mass less than the sensitivity of these instruments.

We recommend some improvements to the semi-automated method. Calculations of dry mass and carbon content are based on several interdependent factors. Dry mass is derived from two biovolume estimates and four conversion

factors. An additional factor is required to estimate carbon content. Considerable variability exists in literature values of wet-to-dry mass and carbon-to-dry mass ratios, along with estimates of meiofauna specific gravity (Table 2). Error in any of these variables propagates to the final calculation. One way that errors might be reduced is to use body volume models and empirically derived values (e.g., carbon content) specific to the organisms under investigation, rather than published conversion factors. Specialized imaging software may also be valuable. For example, harpacticoid measurements in this study relied on eight conversion factors derived by Warwick and Gee (1984) from plasticene displacement models that roughly approximated the shape of animals being measured. The error in these conversion factors when applied to other organisms could be minimized by developing three-dimensional computer models that would accurately measure the volume of organisms given some linear distance measurement, e.g., mid-body width. Matching study specimens with computer models would not only increase the accuracy of volume estimates but also eliminate the time-consuming effort required to apply a color overlay to define image area of individual animals. Inherent inaccuracies in applying these overlays would also be avoided, e.g., variability in axial orientation and angle of the body axis to the photographic plane.

The semi-automated indirect method preserves animals for taxonomic analysis. Another benefit is the ability to measure single individuals, which allows for interspecific biomass measurements, but also makes possible intraspecific measurements of varying life history stages. Both inter- and intraspecific biomass measurements allow for more in-depth studies of energy flux and community interactions. Further development and integration of fully automated graphics and taxonomic identification software (capable of distinguishing organisms on family, genus, or species levels) would greatly increase our ability to measure meiofaunal biomass and lead to greater understanding of ecological interactions.

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