

# Technical Note: The Use of Laser Diffraction Particle Size Analyzers for Inference on Infauna-Sediment Relationships

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**Abstract** For over a century, ecologists and evolutionary biologists have investigated the association between sedimentary characteristics and the infaunal communities inhabiting sediments. Relationships between infauna and, specifically, sediment grain size distributions, have provided a common methodology to predict the distributions, composition, and diversity of soft-sediment communities. Wet/dry sieve methods have traditionally been used to determine grain size distributions, but laser particle size analyzers are becoming increasingly popular and have been shown to measure sediment grain size distributions more efficiently and more accurately than wet/dry sieve methods. An additional, but underexplored, advantage of laser particle size analyzers is their ability to provide uncommonly reported or alternative grain size statistics that can be used to estimate sediment characteristics that are not easily measured using sieve techniques. In particular, measures of sediment heterogeneity are arbitrary and tedious to measure with previously used sieve and microscope techniques. Here, we propose that grain size coefficient of variation, measured using a particle size analyzer, is an improved metric for sediment heterogeneity. We show that grain size coefficient of variation is related to infaunal richness in intertidal habitats along the northern Gulf of Mexico matching previous results relating sediment

heterogeneity to infaunal richness. We discuss the benefits and drawbacks of particle size analyzers and how the use of alternative metrics from laser particle size analyzers may assist the field of benthic ecology.

**Keywords** Gulf of Mexico · Intertidal · Sediment characteristics · Diversity · Habitat · Sediment statistics

## Introduction

The characteristics of the sediment in which infauna live have long been linked to the richness and composition of infaunal communities (For reviews, see Gray 1974; Snelgrove and Butman 1994). The distribution of grain size in the sediment is frequently an important explanatory variable for the community richness, composition, and distribution of infauna (Snelgrove and Butman 1994; Ellingsen 2002; Anderson 2008). Traditionally, in benthic ecology, grain size measures have been made using wet/dry sieving of the sediment (Folk 1974). However, laser diffraction particle size analyzers (hereafter, particle size analyzers) are increasingly being used to derive the grain size characteristics of sediment. Particle size analyzers use the patterns of a laser beam diffracting off particles to infer the dimensions of the particles and thereby producing the grain size distribution from a sample of sediment. The use of particle size analyzers has many advantages over the use of the wet/dry sieving method, including reduced processing time, less biased estimates of grain size distribution, a continuous rather than binned grain size distribution, and greater explanatory power of the variation in macrofaunal assemblages (Eshel et al. 2004; Rodriguez and Uriarte 2009; Forde et al. 2012). Although many studies have compared the results of the two methods using different sediment types, treatments, etc. (see Forde et al. 2012 and Citations within), few studies have recognized that particle size analyzers can

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accurately and more efficiently provide less commonly reported statistics based on particle size distributions that may be important in infauna-sediment relationships.

Previous studies (Whitlatch 1981; Etter and Grassle 1992) found significant relationships between sediment heterogeneity and the richness of infaunal communities in the intertidal and deep sea. Whitlatch (1981) measured sediment heterogeneity by dividing the sediment into arbitrary size classes, enumerating the particles in each size class under a microscope, and calculating Shannon's diversity index. Similarly, Etter and Grassle (1992) divided the sediment into standard geological phi classes, weighed the sediment in each size class, and again calculated Shannon's diversity index. Here, we use the coefficient of variation, as measured by a laser particle size analyzer, as a metric of sediment heterogeneity; a statistic that we suggest is less arbitrary than those previously used. The grain size coefficient of variation is computed as the standard deviation (or "sorting" in geology) of the sediment distribution divided by the mean. The coefficient of variation provides a normalized measure of the variation and thus allows for better comparison of variation across samples with largely different means, a common feature of benthic sediments, and should provide a useful measure of sediment heterogeneity. We investigate whether this metric of sediment heterogeneity is associated with the richness of intertidal infaunal communities along the Gulf of Mexico coast and then provide a discussion on the use of laser particle size analyzers in benthic ecology.

## Methods

### Study Sites

We collected samples from seven intertidal sites along the northern Gulf of Mexico. The sites from west to east were the following: Broussard's Beach in Cameron Parish, Louisiana (29.766° N, 93.283° W CAM); two sites at Elmer's Island, Louisiana (29.131° N, 90.195° W ELMBB and 29.195° N, 90.073° W ELMS); Waveland Beach, Mississippi (30.383° N, 88.81° W WAVE); Ocean Springs, Mississippi (30.383° N, 88.81° W OS); and two sites at Dauphin Island, Alabama (30.253° N, 88.199° W DIS and 30.250° N, 88.199° W DIP). These sites were selected to represent common intertidal sedimentary habitats along the Gulf of Mexico coast. CAM and ELMS are fine sand beaches, ELMBB and DIP are back-bay areas with fine and coarse-grained sediments, respectively, WAVE is a medium-grained sandy beach which was last nourished in 2008, and DIS is a coarse-grained beach. All sites are located near areas of freshwater input and have variable salinity dependent upon weather conditions, recent rainfall events, and the amount of freshwater flow provided by nearby rivers and bays.

### Field and Laboratory Methods

We collected sediment cores from the mid-intertidal (the area regularly inundated under normal tidal cycles and weather conditions) at each site to assess sediment characteristics and infaunal macroinvertebrate richness. Ten cores, approximately 2–3 cm apart from each other, were collected from each site except for ELMS, where only nine samples were collected due to the loss of one sample. All samples were collected between March 24, 2012 and April 6, 2012, using a PVC hand-corer 10 cm in diameter and 5 cm deep. Cores were placed into glass jars and kept on ice until they were returned to the laboratory where they were stored at –30 °C.

In the laboratory, the sediment in each core was manually homogenized. Sediment (~30 g) from each core was compiled into a composite sample that was placed into a freezer until the sediment was characterized. Afterwards, the remaining sediment in the samples was sieved through a 500- $\mu$ m mesh. The material remaining on the mesh was placed into 95 % ethanol with 10 % Rose Bengal dye added to facilitate sorting. Invertebrates were sorted from the sediment under a dissecting microscope and identified to family except for members of Nemertea and Platyhelminthes, which were identified to phylum, and Bivalvia, Gastropoda, and Oligochaeta, which were identified to class. Many of these invertebrates could not be identified to lower taxonomic levels due to varying taxonomic certainty, so we attempted to reduce complications in interpretability by using consistent levels of classification.

The composite sediment sample was analyzed for the particle size distribution. Grain size coefficient of variation was determined for each site using laser diffraction. Ten milliliters of 30 % hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and 5 ml of sodium hexametaphosphate (Na(PO<sub>3</sub>)<sub>6</sub>) were added to approximately 1 g of sediment in a beaker. The addition of hydrogen peroxide oxidized organic matter in the sediment, while the addition of sodium hexametaphosphate acted as a deflocculant for silt and clay particles. The samples were then sonicated for 60 s and analyzed by an LS 13 320 Laser Diffraction Particle Size Analyzer (Beckman Coulter Inc., Brea, CA, USA) for the particle size distribution from which the coefficient of variation in grain size for each site was measured.

### Statistical Methods

To examine the relationship between richness and sediment heterogeneity, we used simple linear regression with the natural log of the sediment coefficient of variation as the independent variable and infaunal taxonomic richness as the dependent variable. Statistics were performed in R (v2.14.2; R Development CoreTeam 2012).

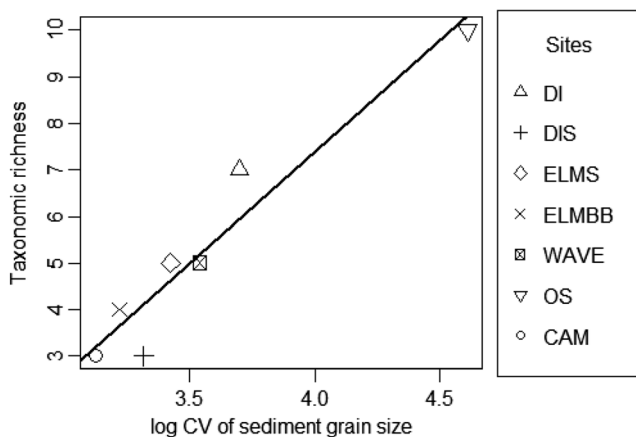
## Results

Grain size coefficient of variation differed among the sites consistent with coarse sediment properties. OS has sediment consisting of both medium-grained sand and high silt and clay content and consequently had the highest grain size coefficient of variation. Similarly, DIP consists of sediment containing both large-grained sand and finer grained particles trapped by algal mats leading to the second highest grain size coefficient of variation. The remaining sites were more homogenous in their coarse sediment characteristics and thus had lower grain size coefficients of variation.

Across the 69 samples, we collected and identified invertebrates representing 5 phyla, 7 classes, and 15 families. Taxonomic richness ranged from a low of 3 to a high of 10. Richness was highest at OS followed by DIP, ELMS, and WAVE, which had the same value, and finally, ELMBB, and DIS, and CAM, which had the lowest richness. Taxonomic richness was highly associated with the log coefficient of variation in sediment grain size (linear regression;  $r^2=0.93$ ,  $p=0.0005$ , 95 % CI on slope (3.23, 6.36); Fig. 1).

## Discussion

We found a strong relationship between sediment grain size coefficient of variation and taxonomic richness that is consistent with previous results examining sediment heterogeneity and infaunal richness (Whitlatch 1981; Etter and Grassle 1992). This relationship provides an example of how metrics that were previously tedious and difficult to measure, but have important links to sediment-infauna relationships, can be more easily measured with particle size analyzers. Another potentially useful measure of sediment heterogeneity that may be



**Fig. 1** Taxonomic richness versus log sediment coefficient of variation for seven sites along the northern Gulf of Mexico. Simple linear regression of the two variables was significant and explained 93 % of the variation in taxonomic richness ( $r^2=0.93$ ,  $p=0.0005$ , 95 % CI on slope (3.23, 6.36))

derived from particle size analyzers is the coefficient of dispersion which is the nonparametric equivalent to the coefficient of variation and is calculated as the interquartile range divided by the median.

Alternative grain size statistics measured using particle size analyzers are also available that may assist benthic ecologists. For example, we know that many deposit feeding invertebrates are size selective with respect to the sediment and organic material that they consume (Whitlatch and Obrebski 1980; Stamhuis et al. 1998; Guieb et al. 2004). A particle size analyzer can readily provide the percentage of sediment within a predefined range (e.g., a deposit feeder's selective range), whereas most sieve sets are generally related to geological phi classes, which would not easily measure the amount of sediment within a particular, more biologically defined, range. The percentage of sediment within a deposit feeder's selective range could provide an important predictor for their presence and abundance and could help in modeling the ranges of ecologically and economically important benthic organisms (Anderson 2008). Furthermore, because particle size analyzers only need approximately 1 g of sediment to conduct measurements, they should be particularly useful for measuring fine spatial scale grain size distributions, for example, within a single core or along a transect. Particle size analyzers could also be used for fine temporal scale grain size distributions in studies using sediment traps, which are commonly used to understand relationships between the dynamics of depositional environments and community composition.

Although particle size analyzers offer several advantages over the wet/dry sieve methods, they do have some drawbacks. First, the particle size analyzer itself can be orders of magnitude more expensive than a sieve set and, unfortunately, the economy of research equipment is often a major consideration. Another disadvantage is that most particle size analyzers can only measure particles with a diameter less than 2,000  $\mu\text{m}$ . Therefore, sieves must be used to analyze the larger particle fractions for sediment with particles larger than 2,000  $\mu\text{m}$ . Lastly, several methods have been developed for pretreatment of the sediment before analysis. The best pretreatments for achieving measures of sediment characteristics that match those actually experienced by the organisms is still under investigation and, notably, may not be the same treatments commonly used by geologists (Snelgrove and Butman 1994; Forde et al. 2012).

Overall, laser particle size analyzers, despite their drawbacks, offer a useful tool for benthic ecologists exploring infauna-sediment relationships. The fact that particle size analyzers can quickly process samples and give more quantitatively accurate results makes their use promising for the future of relating sediment characteristics to the distributions of infauna (Rodriguez and Uriarte 2009; Forde et al. 2012). Furthermore, as we show here, the ability of laser particle size analyzers to more easily provide alternative and uncommonly

reported statistics on sediments increases their potential impact on the field of benthic ecology.

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